UNRAVELING THE ROLES OF GENOTYPE AND ENVIRONMENT IN THE EXPRESSION OF PLANT DEFENSE PHENOTYPES

Abigail S. Potts

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Department of Ecology and Evolutionary Biology
University of Michigan

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Advisor: Mark D. Hunter

Committee: Regina S. Baucom, Inés Ibáñez

Abstract

Phenotypic variability, resulting from the combination of genetic information and environmental conditions, is a major driver of ecological interactions. However, determining what aspects of phenotypic variation are due to genetics, the environment, and their interaction is critical to understanding ecological interactions and their evolutionary consequences. To assess how genetic variation and local environmental variation act as drivers of phenotypic variation in the context of plant-herbivore interactions, we combined a series of common-garden experiments with data collected from a naturally growing population of *Asclepias syriaca*, common milkweed, from which the common garden seeds originated. Specifically, we compared phenotypes of 14 maternal lines in two common gardens with their 14 maternal genets and carried out a reciprocal transplant experiment with three of the maternal lines. These trials allowed us to identify genetic variation in traits of common milkweed and then compare the traits with those of the natural population of milkweed to assess how local growing environment dictates trait expression. We measured plant growth, defoliation, and arthropod abundances and sampled leaves for their cardenolide concentrations, latex exudation, and carbon and nitrogen concentrations. Our results suggest that milkweed foliar quality and milkweed-herbivore interactions are mediated by a combination of genetic variation and response to the local environment. We observed that milkweed resistance to chewing herbivores was genetically variable but appeared mediated by the local growing environment. We recorded that one type of milkweed defense, foliar cardenolides, was environmentally controlled and lacked genetic variation within our population of maternal lines. In contrast, we observed significant genetic variation among maternal lines in foliar latex exudation. Some variation in milkweed-aphid interactions was best explained by local environment while some variation remained unexplained. Strikingly, any genetic variation detected in a common garden was uncorrelated with the other common garden and this variation was unrelated to the naturally growing maternal genets. This indicates that even when genetic variation is present, environmental variation is the dominant driver of trait expression in this population of common milkweed. Our data also suggest that milkweeds are phenotypically plastic, with unknown consequences for their fitness under environmental change. This research aids in understanding which milkweed traits may be acted upon by natural selection and may therefore be most likely to adapt to the rapid environmental change seen in the modern world.

Introduction

Phenotypic variability, resulting from both genotype and environment, drives many ecological and evolutionary processes (Via & Lande 1985; Hahn *et al.* 2019; Zirbel & Brudvig 2020). To understand the significance of an organism's phenotype, and consequently its interactions with organisms around it, we must understand the independent and interactive effects of both the organism's genotype and its environment in determining that phenotype. Genetic variation is well known to have substantial effects on trait expression (Agrawal *et al.* 2002; Agrawal & Hastings 2019a), providing the variation on which selection may act during evolution (Burger & Lynch 1995; Baucom & Mauricio 2004). Environmental variation, in addition to its role in contributing to phenotypic variation (Davis *et al.* 2005; Couture *et al.* 2015; Decker *et al.* 2019), is also a source of strong selection on certain phenotypes, thus influencing which genotypes may prosper (Vannette & Hunter 2011; Jay *et al.* 2012; Beemelmanns & Roth 2017). When environments begin to change, traits may become mal-adapted (Ibáñez *et al.* 2010; Jay *et al.* 2012; Patankar *et al.* 2013; Sorte *et al.* 2013), and, if the population lacks genetic variability, the threat of extinction may become a reality (Burger & Lynch 1995).

A substantial amount of phenotypic variation exists within and among species and this variation influences species interactions (Coley 1987; Wetzel *et al.* 2016, 2018; Bucharova *et al.* 2017; Agrawal & Hastings 2019b). However, the source of this variation is not always clear. Understanding *wh*y species exhibit trait variation is critical to understanding ecological interactions and evolutionary consequences. Plant-herbivore interactions provide interesting systems for investigating the causes and consequences of phenotypic variation because they are ubiquitous in terrestrial systems and mediate numerous indirect effects with other herbivores (Ali & Agrawal 2014), pollinators (Moreira *et al.* 2019), soil microbial associates (Peschel *et al.* 2015) and other trophic levels (Price *et al.* 1980; Hunter 2016). Because plants are sessile during significant portions of their lifecycles, they experience strong selection to adapt to their local environment (Cipollini 2002; Bossdorf *et al.* 2005; Jay *et al.* 2012; Weißhuhn *et al.* 2012; Bucharova *et al.* 2017), including in defense traits against their herbivores (Coley 1987; Agrawal & Van Zandt 2003; Agrawal 2005; Vannette & Hunter 2011).

Plant defenses can be heritable (Wooley *et al.* 2007) but also determined by environmental conditions (Mondor *et al.* 2006; Ode *et al.* 2014; Decker *et al.* 2018; Hahn & Maron 2018), including insect attack (Howe & Schaller 2008; Ali & Agrawal 2014). However,

plant responses to environmental conditions are dependent on genetic information (Des Marais *et al.* 2013; Lehndal & Ågren 2015). The interplay between genotype and environment (genotype-by-environment interactions; G x E) is well-known to drive ecological interactions (Vannette & Hunter 2011; Des Marais *et al.* 2013; Saltz *et al.* 2018). However, it is often difficult to untangle whether genetics or the environment are responsible for phenotypic variation (Maddox & Root 1987; Muola *et al.* 2010).

In contrast to plants, mobile herbivores are able to make choices about their location and food source (Murphy & Loewy 2015; Jones & Agrawal 2019). Evolutionary or ecological variation in plant defenses against herbivory are therefore countered in part by herbivore choices – an herbivore can lower consumption in response to increased toxicity (Whitehead & Poveda 2011; Whitehead *et al.* 2016), continue to consume the plant but face consequences of decreased health and fitness (Tao *et al.* 2016), or even sequester the toxin for its own purposes (Petschenka & Agrawal 2015; Jones *et al.* 2019). If environmental conditions change, thereby changing plant defense traits, we might expect herbivory patterns to change as well (Ode *et al.* 2014).

Anthropogenic climate change is devastating global insect populations (Brower *et al.* 2012; Hallmann *et al.* 2017), and this has effects on overall ecosystem function (Hallmann *et al.* 2017; Lister & Garcia 2018). When environmental conditions change rapidly, population adaptation depends on natural selection to act on heritable genetic information (Via & Lande 1985). Determining the proportion of phenotypic variation that is attributable to genetic factors allows for better prediction of the ability of populations to adapt to a rapidly changing environment. In this study, we assess how genetic variation and local environmental variation act as drivers of phenotypic variation and plant-herbivore interactions in a naturally growing population of *Asclepias syriaca*, common milkweed.

System of Study

Milkweeds (Apocynaceae) and their herbivores have become a model system for studying the ecology and evolution of plant-herbivore interactions (Brower *et al.* 1968; Malcolm 1994; Zehnder & Hunter 2007; Hahn *et al.* 2019; Meier & Hunter 2019). Milkweed grows clonally in genetic individuals (genets) (Woodson 1954) and these genets resist herbivory through several defensive traits: cardenolides, a group of cardiac glycoside steroids (Malcolm 1991), latex, a sticky substance that inhibits chewing herbivores (Zalucki & Malcolm 1999), and trichomes,

hair-like structures that inhibit herbivore feeding (Levin 1973; Agrawal 2004a). A group of specialist herbivores, including the monarch butterfly and several aphid species, have evolved to overcome milkweed's defenses (Agrawal 2012; Sternberg *et al.* 2012; Ali & Agrawal 2014; Birnbaum & Abbot 2018). These specialized interactions provide a unique model for the study of plant-insect interactions. Milkweed displays both inter- and intra-specific variation in defensive phenotypes (Zehnder & Hunter 2007; Agrawal & Hastings 2019b; Hahn *et al.* 2019), which in turn influence their ecological interactions (Zalucki *et al.* 1990; Birnbaum & Abbot 2018). Global environmental change has greatly impacted the ecology of milkweeds (Malcolm 2017) and their specialized herbivores (Pleasants & Oberhauser 2013; Decker *et al.* 2018). Understanding the relative contributions of environment and genotype to milkweed phenotype may allow predictions of how, and how rapidly, milkweed and its specialist herbivores may adapt to their changing environment.

To determine if milkweed trait variation is due more to genetic variation or local environmental conditions, we grew *Asclepias syriaca* in three experiments: a field common garden, a greenhouse common garden, and a reciprocal field transplant. We compared the data from the common gardens with data from the local population of *A. syriaca* from which the common garden seeds originated (referred to as "maternal genets" hereafter). M.D. Hunter has studied the native *A. syriaca* population at the University of Michigan Biological Station for the past 12 years, recording data on milkweed physical and chemical defense traits and milkweed-associated insect population density for multiple genetic individuals. Because each genet has experienced just one location in space, the available data cannot separate genetic effects from effects of the local environment on phenotype. We compared results from common garden experiments with those from the natural population to assess the relative contributions of genotype and environment to plant growth, defense, and insect attack. Common gardens create a single environment in which plants are grown, and therefore any differences among groups are due only to genetic variation (Cipollini 2002; Agrawal & Van Zandt 2003; Pellissier *et al.* 2016). Reciprocal environmental transplants allow assessment of local environmental adaptation (Bucharova *et al.* 2017) by comparing phenotypes of local and non-local genotypes in one location. By employing these three experiments and comparing the results to the same data collected from the unmanipulated native population, this study assesses the contributions of

genotype and environment to the expression of defense within a plant population under regional decline.

Here, we assess the following predictions: (1) If milkweed phenotypic variation is due predominantly to genetic variation, common garden plants will express variation among maternal lines in foliar chemistry and plant growth traits that will correlate strongly with those measures in the unmanipulated maternal genets. (2) Conversely, if phenotypic variation is largely due to differences in local environment, common garden plants will show low genetic variation in foliar chemistry and growth traits, and such traits will be uncorrelated with those of the unmanipulated maternal genets. (3) If environment is a dominant driver of phenotypic variation, then all plants in one reciprocal transplant location will show similar phenotypes regardless of their maternal line. (4) In contrast, if the traits expressed by the reciprocal transplant milkweeds are more similar among maternal lines than among transplant locations, it suggests a stronger genetic than environmental contribution to phenotypic variation.

By combining data collected from two common gardens, a reciprocal field transplant, and a native milkweed population, we are able to assess the contributions of genetic variation and local environmental variation to variation in milkweed phenotype, and consequently better understand the potential for population adaptation.

Methods

Overall Experimental Design

To understand how milkweed genotype and environment influence growth and defense phenotypes, we designed three experimental groups: a field common garden, a greenhouse common garden, and a reciprocal field transplant (Figure 1a). The field common gardens allowed us to detect genetic variation in this population of common milkweed, while the reciprocal field transplant allowed us to estimate the effect of local environment on the phenotypic expression of common milkweed. The seeds for all experiments came from fourteen spatially mapped genetic individuals (genets) of *Asclepias syriaca,* common milkweed, growing at the University of Michigan Biological Station (UMBS) in Pellston, MI (45.558605, -84.677488). The seeds collected from the fourteen genets were at least half-siblings (multiple seed pods from unknown fathers for each genetic mother). Seeds and seedlings were classified by their maternal genotype and are referred to as “maternal lines” hereafter.

The field common garden and greenhouse common garden were both randomized block designs, containing the same 14 *A. syriaca* maternal lines grown in 18 blocks. There was one individual of each maternal line in each block, with the position of the maternal lines randomized in each block. The same experimental set up was replicated in the field and greenhouse. The reciprocal field transplant consisted of three maternal lines, each grown "at home" and "away". At each of the 3 maternal locations, all three maternal lines were grown in soil from that maternal location (i.e. maternal location includes the maternal soil). Therefore, at each maternal location, we grew offspring plants from the "matching" maternal line and two "non-matching" maternal lines (Figure 1b).

We measured plant growth rates, defoliation, and arthropod abundances weekly from all common garden plants (12 weeks from June 3rd – August 21st) and reciprocal transplant plants (10 weeks from June 19th – August 21st) and monthly from the native maternal genets. We collected foliar chemistry from all plants once in mid-July, in the middle of the growing season (methods below).

Maternal Genet Sampling

To measure trait variation in the naturally growing milkweed population, we sampled 5 individual ramets from each of the 14 maternal genets (70 ramets total) on three dates (mid-June, mid-July, and mid-August, 2019). We measured plant growth (height, leaf number, stem diameter), defoliation, and arthropod abundance for each ramet. We collected foliar chemistry samples and measured latex exudation once in mid-July.

Estimate of Defoliation

To assess the contributions of genetic variation and environment to plant resistance to herbivory, we estimated defoliation by chewing herbivores from each plant in the common gardens, the reciprocal transplant experiment, and the maternal genets. We visually categorized each leaf longer than 1 cm into one of the following defoliation levels: no defoliation, 0-5%, 5-30%, 30-50%, 50-70%, 70-90%, >90% defoliated. To estimate the overall percentage of defoliation per plant, we multiplied the number of leaves in each defoliation level by the median value of the level (2.5, 17, 40, 60, 80, 95), and added the values. This sum was then divided by the total number of leaves on that plant. The final value represents the overall estimation of percent

defoliation for that plant. This method has a long history in the literature, and correlates strongly with independent estimates of defoliator activity (Hunter 1987; Hunter *et al.* 1997; Meier & Hunter 2019).

Common Gardens

Growing the Plants

Plants for the field and greenhouse common gardens were grown from seed for one month at the University of Michigan in Ann Arbor before transfer to UMBS. Seeds were cold stratified for 6 weeks, treated with household bleach (5%), germinated in petri dishes for one week, and then planted in Sungro Metro-mix® 360 potting soil in Deepots®. Seedlings were grown in a controlled growth room (14:10 L:D, mean temperature 78°F) for the month of April 2019. Seeds were planted in April to ensure that plants were large enough to withstand field conditions by June, when local ramets emerge. We transported plants from Ann Arbor to UMBS on May 1st, 2019 to complete an additional month of greenhouse growth while outside conditions were still too cold. Plants were then either left in the greenhouse (greenhouse common garden) or transferred outside to the field common garden on June 1st, 2019. This timing matches the typical phenology of the local milkweed population at UMBS (M.D. Hunter, *personal communication)*.

Experimental Setup

The randomized block design of 18 blocks, each containing one individual of 14 maternal lines resulted in 270 plants total per common garden. Each plant was grown in an 18cm x 16cm pot held on benches (greenhouse) or set into the ground such that the topsoil of the pot was level with the ground (field). Each block consisted of two rows of 7 plants. Within each block of the field common garden, plants were spaced 1 m apart and 1.5 m separated each block. A 12.68 m x 32.53 m fenced exclosure surrounded the field common garden to protect plants from deer and rabbit browsing. The greenhouse common garden plants were arranged on benches so that plants were not touching. Plants were watered *ad libitum* and fertilized using Osmocote controlled release fertilizer (14:14:14 N:P:K) (ICL Specialty Fertilizers, Dublin, OH) once in May and once in July. From each *A. syriaca* plant we measured weekly arthropod abundance and plant height, leaf number, defoliation, and base stem diameter (12 weeks total). In mid-July, we

measured foliar latex exudation and collected tissue to measure foliar concentrations of cardenolides, carbon, and nitrogen.

Reciprocal Transplant Experiment

We chose three of the 14 maternal genets to provide seed for reciprocal field transplants. We chose genets that spanned the known range of nutritional quality in *A. syriaca* at UMBS and also originated from spatially separated locations within the UMBS population. Based on the past 10 years of sampling, genet 14 has high foliar nitrogen concentrations (3.596% N, 42.964% C), genet 20 has low foliar nitrogen concentrations (2.719% N, 43.003% C), and genet 44 has high foliar carbon concentrations (2.990% N, 44.341% C) (M.D. Hunter, *unpublished data*). Seeds of each of the three maternal lines were planted in the soil and location of all three of the original maternal genets.

Seeds were planted in 18cm x 16cm pots on May 13th, 2019 at UMBS in the soil of their reciprocal transplant destination. Seedlings were grown in the greenhouse until large enough to withstand outside conditions and were placed in the field on June 19th. Replicate seeds per maternal line were established within the spatial boundaries of each of the maternal genets (Figure 1b). Each transplant location (maternal genet location) hosted 5 replicate plants of each of 3 maternal lines, totaling 15 plants at each maternal genet location (45 plants total in the experiment). Therefore, each maternal genet location hosted offspring plants of each of the three maternal lines (including the matching line). All plants in a given location were therefore in the same soil and environment of the "host" maternal genet. We randomized the plants at each of the three locations. Plants were protected by a wire open-top cage to block deer and rabbit browsing but allow insects to access the plants. We measured arthropod abundance and plant height, leaf number, and defoliation weekly (10 weeks from June 19th – August 21st) for each *A. syriaca* plant; foliar chemistry samples were collected once on August 21st. Stem diameter was not measured due to the small size of plants.

Plant Chemical Analyses

Due to time constraints, chemical analyses (cardenolides, C:N) were performed on foliar samples from only half of the blocks (10 – 18) in the two common gardens. We analyzed foliar chemistry in July because insect diversity and density are highest during July and this month represents the

time period during which milkweed chemistry is most likely responsive to plant-herbivore interactions (Agrawal 2004a) (Appendix A, Table A1, Figure A1).

To analyze foliar cardenolide concentrations, we cut 6 leaf disks with a hole puncher from the fifth leaf pair of each plant and placed the disks in 1 mL of methanol. Samples were stored at -10 °C for later cardenolide analysis. We took 6 additional disks from the same leaves to estimate the dry mass of the cardenolide samples. To extract cardenolides, we finely ground the leaf disks in methanol, sonicated the mixture for 1 hour at 60 °C, and centrifuged for 6 minutes. We transferred the supernatant to new 1 mL Eppendorf tubes and evaporated the samples under vacuum at 45 °C until dry. We resuspended the sample in 300 mL of methanol and used reverse-phase ultra-performance liquid chromatography (UPLC) on a Waters Acquity UPLC with an Acquity BEH C18 column (1.7 μm, 2.1 x 50 mm, Waters Inc., Milford, MA, USA). We separated and quantified cardenolides with a 0.15 mg/mL digitoxin internal standard (Sigma Chemical Company, St. Louis, Missouri, USA). Each 2 μL injection sample was eluted for 9 minutes at a constant flow rate of 0.7 mL per minute under a mobile phase of 20% acetonitrile (ACN): 80% water for 3 minutes followed by a gradient increasing to 45% ACN: 55% water over the remainder of the run. Cardenolides were quantified using a diode array detector scanning between 200 and 300 nm and we identified cardenolides as peaks with symmetrical absorbance between 216-222 nm. To calculate cardenolide concentrations, we took the sums of all separated peak areas, corrected by the concentration of the internal digitoxin standard and estimated by the dry sample mass.

We measured milkweed latex exudation by collecting latex from the 6 holes cut for the cardenolide samples on pre-weighed paper disks. Disks were dried in a drying oven at 45º C for 24 hrs and then weighed. We measured latex exudation in all 18 blocks in both common gardens.

To analyze foliar carbon and nitrogen concentrations, we collected 2-3 leaves from each plant. Leaves were dried in a drying oven at 45º C and finely ground. Leaf powder was dried again for 24 hrs before 2 μg of each sample was transferred to a tin capsule. Carbon and nitrogen concentrations were measured on a ThermoScientific EA 1112 elemental analyzer. We used 99.7% caffeine powder as an external standard.

Statistical Methods

Statistical analyses were performed using SAS version 9.4 unless noted otherwise.

Plant Growth and Herbivore Resistance

We used generalized linear mixed models (proc glimmix) to assess genetic variation in plant growth and resistance to herbivores (defoliation level). For the common garden analyses, we included block and individual plant ID (nested within maternal line and block) as random variables to account for repeated measures from individual plants and any effects of autocorrelation within blocks. Week was a continuous variable while plant ID, maternal line, and block were class variables. Because plants began to senesce and reduce in size by the end of the season, our models included a quadratic term for time. Overall, our models assessed the effects of maternal line on variation in plant growth (height, leaf number, average base stem diameter) and resistance (defoliation) over the growing season. Significant interactive effects of maternal line and week on character traits represent genetic variation in rates of growth or resistance to herbivory.

Because we collected data from maternal genets only once each month, we used month as a class variable in analyses of variation in growth and resistance of maternal genets. Otherwise, we followed the same model structure as above without a block term.

To analyze data from the reciprocal transplant experiment, we used a similar glimmix model structure but removed the random block term and assessed the effects of maternal line, week, transplant location, and their interactions on plant traits.

Insect Populations

Most insect species were encountered too rarely to analyze separately, and aphids were by far the most abundant herbivores that we encountered (Appendix A, Table A1). Accordingly, we restrict our analyses of insect abundance to aphids. However, many individual milkweed plants were never colonized by aphids. Therefore, we first analyzed variation in aphid populations among milkweed maternal lines or genets by performing a generalized linear mixed model (proc glimmix) using a binomial distribution with a logit link function to assess aphid presence/absence on plants. This model worked well for one aphid species, *Aphis asclepiadis,* but would not converge for the second species, *Myzocallis asclepiadis*. Therefore, we used a generalized linear model (proc genmod) with a binomial distribution and logit link function for *M. asclepiadis*. Because proc genmod in SAS does not recognize random effects, we designated

plant ID (nested within maternal line and block) as a repeated effect and accounted for variation among blocks by assigning block as a main effect.

For those plants which hosted aphids, we then used a mixed model (proc mixed) and log transformed aphid population counts to assess genetic variation for resistance to aphids among maternal lines over time. We held block and plant ID (nested within block and maternal line) as random variables and used plant ID (nested within block and maternal line) as the repeated subject term.

We recorded *Aphis asclepiadis* on only 13 maternal ramets throughout the season, but only two of the six maternal genets represented had three or more replicate ramets. Therefore, we did not have enough representation to perform meaningful statistical tests for *A. asclepiadis* population growth among maternal genets. *Myzocallis asclepiaidis* appeared only in the month of August and appeared on 23 maternal ramets. Five of the nine maternal genets represented had three or more ramets with aphids, and therefore we restrict our analysis to those 5 maternal genets (17 ramets). We log-transformed *M. asclepiadis* numbers and examined differences in *M. asclepiadis* populations among maternal genets using a general linear model (proc glm). Because *M. asclepiaids* only appeared in August, no month term or repeated measure was required.

Plant Chemistry

To assess genetic variation in foliar chemistry traits (carbon, nitrogen, cardenolides, and latex), we used a generalized linear mixed model (proc glimmix) and held plant ID, maternal line, and block (for common gardens) as class variables and maternal line and plant ID (nested with maternal line and block) as random variables. We log-transformed cardenolide data prior to analysis to meet assumptions of homogeneity of variance. For the maternal genets we used a general linear model (proc glm) and held plant ID and maternal line as class variables. Because we only used chemistry data from one date (mid-July), no week term was required.

Associating Genetic Variation Estimated in Common Gardens with Trait Variation in the Field

A major goal of our study was to use estimates of genetic variation measured in the common gardens to assess sources of trait variation in our natural field population of milkweed. To accomplish this, we correlated traits (growth, chemistry, resistance) measured in each of the common gardens with those same traits measured in the natural field population of maternal

genets. We also correlated these traits between the two common gardens (field and greenhouse) to assess the consistency of our estimates of genetic variation between the two types of common garden. We first calculated average trait values for each maternal line at each location (both common gardens and the maternal genets). We then calculated the slopes of the regressions for each trait across locations, using the means (14 genets/maternal lines) as data points. Regression statistics were calculated using Excel for Mac version 16.33.

Because plants were senescing by the end of the season (Appendix B), we calculated initial growth rates of our milkweeds between weeks one and six for the common gardens and between mid-June and mid-July for the maternal genets. Week 6 of the common garden experiments was the same week as the mid-July sample of the maternal genets. We calculated initial growth rate for each individual plant (separately for height, leaf number, and diameter) using the following formula: (Week 6 data – Week 1 data) / (Week 1 data) = Initial Growth Rate. We averaged the initial growth rates for each maternal line/genet and compared as described above.

To estimate sources of variation in resistance to herbivory in the natural field population of milkweed, we first correlated defoliation between the field common garden maternal lines and their associated maternal genets. We calculated average defoliation values for August for each maternal line/genet from the field common garden and the maternal genets. We used the data from August because most defoliation occurred in August. We calculated the slopes of the regressions for defoliation between the field and maternal genets, using the means (14 genets/maternal lines) as data points. Regression statistics were calculated using Excel for Mac version 16.33.

In addition, we assessed sources of genetic variation in resistance to herbivory by correlating foliar cardenolide concentration and latex exudation (separately) between both common gardens and the maternal genets. We followed the same method as defoliation above but used the chemistry data collected in July.

Finally, we explored potential genetic tradeoffs between milkweed growth and resistance to herbivores (Strauss & Agrawal 1999; Züst *et al.* 2015). We used Principal Components Analysis to generate a single PCA axis for growth (separately for each common garden & the maternal genets). That is, we combined the initial growth rates of height, leaf number, and stem diameter into a single PCA axis for each common garden/maternal population. We then assessed

correlations among milkweed resistance and growth traits (within maternal lines of each common garden and within the maternal genets). We calculated pair-wise correlation coefficients among the growth PCA axis, and foliar cardenolide concentrations, latex exudation, foliar C:N ratios, and defoliation.

Results

Drivers of Variation in Defoliation

Maternal lines grown in the field common garden expressed genetic variation for resistance, accumulating defoliation at different rates (Week*Maternal line, $F_{13, 2746} = 2.30$, $P = 0.0051$, Figure 2a). Similarly, the maternal genets also varied in resistance, accumulating defoliation at different rates (Maternal genet*Month, $F_{26, 110} = 6.56$, $P < 0.0001$, Figure 2b). However, defoliation of the maternal lines in the field common garden was uncorrelated with defoliation experienced by their naturally-growing maternal genets ($y = -0.146x + 4.2767$, $R^2 = 0.0056$, $P = 0.7992$), suggesting that there may be determinants of resistance in addition to genetic variation, for naturally-growing milkweeds. In support of this, milkweeds in the reciprocal transplant experiment accumulated defoliation at different rates among transplant locations (Week*Location $F_{2, 392} = 22.53$, $P <0.0001$, Figure 3) whereas maternal lines accumulated defoliation at similar rates (Maternal line*Location, $F_{4, 36} = 0.20$, $P = 0.9381$, Figure 3). Overall, these results indicate that local growing environment is an important driver of resistance to chewing herbivores. As expected, defoliation did not exceed 15% in the greenhouse and did not differ among maternal lines (Maternal line, $F_{13, 221} = 0.18$, $P = 0.9994$; Week*Maternal line, $F_{13, 2758} = 1.52$, $P = 0.1007$).

Drivers of Variation in Insect Populations on Milkweed

We observed no evidence for genetic variation in *Aphis asclepiadis* colonization among milkweed maternal lines (Maternal line, $F_{13, 221} = 0.76$, $P = 0.6976$; Week*Maternal line, $F_{13, 2747} = 0.60$, $P = 0.8564$) in the field common garden. Likewise, after colonization, we observed no genetic variation in *A. asclepiadis* population densities among maternal lines (Maternal line, $F_{13, 111} = 1.08$, $P = 0.3859$; Week*Maternal line, $F_{13, 130} = 1.23$, $P = 0.2683$, Figure 4a), indicating that genetic variation may not account for resistance against *A. asclepiadis* population growth.

Population sizes of *A. asclepiadis* were too low on both the maternal genets and the reciprocal transplant milkweeds to provide insight.

As with *A. asclepiadis*, we found no evidence for genetic variation in *Myzocallis asclepiadis* colonization among milkweed maternal lines in the field common garden (Maternal line, $X^2_{13} = 12.61$, P = 0.4783; Week*Maternal line, $X^2_{13} = 15.48$, P = 0.2782). After colonization, we observed no genetic variation in *M. asclepiadis* population levels among maternal lines (Maternal line, $F_{13, 219} = 1.22$, P = 0.2645; Week*Maternal line, $F_{13, 910} = 1.49$, P = 0.1133, Figure 4b). In contrast, we did observe variation among the five maternal genets analyzed in the population densities of *M. asclepiadis* ($F_4 = 5.50$, P = 0.0095, Figure 5), suggesting that local environment may be a significant determinant in *M. asclepiadis* population growth. Unfortunately, population densities of *M. asclepiadis* were too low on reciprocal transplant milkweeds to provide any additional insight.

Drivers of Variation in Plant Foliar Quality

Maternal lines in the field common garden and the greenhouse common garden expressed no genetic variation in foliar cardenolide concentration ($F_{13, 101} = 1.11$, P = 0.3568; $F_{13, 98} = 1.53$, P = 0.1212, respectively; Figure 6a, b). Accordingly, cardenolide concentrations in the field common garden were uncorrelated with those of the greenhouse common garden ($y = 0.216x + 0.1749$, $R^2 = 0.004$, P = 0.8269). In contrast, foliar cardenolide concentrations did vary among naturally growing maternal genets ($F_{13} = 3.42$, P = 0.0007, Figure 6c). However, the foliar cardenolide concentrations of the maternal genets were uncorrelated with those of either the field or the greenhouse common gardens ($y = -0.297x + 0.0584$, $R^2 = 0.1265$, P = 0.2120; $y = 0.0322x + 0.0321$, $R^2 = 0.0167$, P = 0.6592, respectively). Consistent with results from both common gardens, milkweeds in the reciprocal transplant experiment did not vary in cardenolides among maternal lines (Maternal line, $F_2 = 1.20$, P = 0.3316, Figure 7). However, unlike the maternal genets, cardenolide concentrations did not vary among transplant locations either (Location, $F_2 = 0.44$, P = 0.6552; Maternal line*Location, $F_3 = 0.18$, P = 0.9096, Figure 7).

We did not observe genetic variation in foliar nitrogen concentration in the field or greenhouse common gardens ($F_{13, 104} = 1.38$, P = 0.1821; $F_{13, 104} = 1.73$, P = 0.0659, respectively; Figure 8a, b), and foliar nitrogen concentrations between the two common gardens were uncorrelated ($y = 0.262x + 1.759$, $R^2 = 0.0732$, P = 0.3497). However, foliar nitrogen

concentrations varied substantially among the maternal genets ($F_{13, 56} = 11.21$, $P < .0001$, Figure 8c), suggesting that local environment is an important driver of foliar nitrogen concentrations. Accordingly, foliar nitrogen concentration in the maternal genets was not predicted by those of the maternal lines in either of the common gardens (Field, $y = 0.2276x + 1.899$, $R^2 = 0.0082$, $P = 0.7575$; Greenhouse, $y = 0.7734x + 0.6131$, $R^2 = 0.0893$, $P = 0.2993$).

In contrast to foliar nitrogen concentrations, maternal lines expressed genetic variation in foliar carbon concentration in both the field and greenhouse common gardens ($F_{13, 104} = 3.06$, $P = 0.0007$; $F_{13, 104} = 3.46$, $P = 0.0002$, respectively; Figure 9a, b). However, the foliar carbon concentrations of the maternal lines were uncorrelated between the two common gardens ($y = 0.4262x + 25.713$, $R^2 = 0.2491$, $P = 0.0693$), suggesting an interaction between the environment (greenhouse versus field) and the expression of genetic variation in foliar carbon concentration. However, the maternal genets also varied in foliar carbon concentrations ($F_{13, 56} = 3.23$, $P = 0.0011$, Figure 9c), and foliar carbon concentrations in the maternal genets were correlated with both the field and the greenhouse common gardens ($y = 1.195x - 8.6549$, $R^2 = 0.3032$, $P = 0.0413$; $y = 1.5071x - 23.026$, $R^2 = 0.3517$, $P = 0.0254$), suggesting that genetic variation is a major driver of differences in foliar carbon concentrations in field-grown milkweeds.

We observed genetic variation in foliar latex exudation in both the field and greenhouse common gardens ($F_{13, 221} = 3.89$, $P < 0.0001$; $F_{13, 221} = 2.49$, $P = 0.0034$, respectively; Figure 10a, b). However, latex exudation was uncorrelated among maternal lines between the two common gardens ($y = 0.3414x + 0.0019$, $R^2 = 0.108$, $P = 0.2512$) again suggesting an interaction between the environment (greenhouse versus field) and the expression of genetic variation in foliar latex exudation. Maternal genets also varied in foliar latex exudation ($F_{13} = 5.64$, $P < 0.0001$, Figure 10c), but foliar latex in the maternal genets was uncorrelated with either the field or greenhouse common gardens ($y = 0.1645x + 0.0014$, $R^2 = 0.0151$, $P = 0.6756$; $y = 0.4814x + 0.0005$, $R^2 = 0.1396$, $P = 0.1882$, respectively).

Finally, we assessed correlations among milkweed growth and resistance traits within each common garden and within the maternal genets. A table of all correlation results may be found in Appendix A, Table A2. Here, we briefly denote significant results. Growth and foliar C:N ratios in the maternal genets were negatively correlated ($y = -2.1629x + 17.927$, $R^2 = 0.5045$, $P = 0.0044$). Foliar cardenolide concentrations were positively correlated with defoliation of the maternal genets ($y = 208.58x - 5.2871$, $R^2 = 0.8878$, $P = 0.0094$). In the field

common garden maternal lines, latex was negatively correlated with defoliation ($y = -295.3x + 1.1569$, $R^2 = 0.2826$, $P = 0.0504$), and in the greenhouse common garden maternal lines, foliar C:N ratios were negatively correlated with foliar cardenolide concentrations ($y = -0.0341x + 0.7928$, $R^2 = 0.4891$, $P = 0.0054$).

Overall, our results reveal some important patterns that we display in Table 1. Strikingly, maternal genets display significant trait variation, but this variation is unrelated to the genetic variation expressed in the maternal lines in both the greenhouse and field common gardens. Additionally, genetic variation among the maternal lines was uncorrelated between the two common gardens. This suggests that environment is the major driver of phenotypic trait variation in our population of common milkweed, and that common milkweed expresses high levels of phenotypic plasticity.

Plant Growth

The primary goal of our study was to measure genetic variation in resistance and resistance traits in milkweed. However, during our experiments, we also estimated plant size (height, leaf number, diameter) whenever we counted insects. We then analyzed our size estimates using the same techniques described above. Because measuring genetic variation in plant size was not a primary goal of our study, we restrict the presentation of these analyses to Table 1 and Appendix B.

Discussion

Our results suggest that milkweed foliar quality and milkweed-herbivore interactions are mediated by a combination of genetic variation and response to the local environment. These results add to a body of literature describing the complexities of plant trait expression (Maddox & Cappuccino 1986; Muola *et al.* 2010; Bustos-Segura *et al.* 2014) and the resulting interactions with insects (Johnson & Agrawal 2005; Vannette & Hunter 2011; Lehndal & Ågren 2015). Milkweeds display significant phenotypic diversity in defensive traits (Hahn *et al.* 2019), but phenotypic diversity results from interactions between the local growing environment and the genetic profile. When genetic variation and environment interact (genotype-by-environment interactions (G x E) (Saltz *et al.* 2018)), genetic groups express different phenotypes in response to variation in environmental conditions (Smith & Kruglyak 2008; Botwright Acuña & Wade

2012; Aslam & Karanja 2020). The consequences of this G x E phenotypic trait variation may contribute to species adaptation to rapid environmental change (West-Eberhard 1989; Des Marais *et al.* 2013). Therefore, we must understand how and why milkweed express trait variation to predict the adaptation potential of milkweed and its specialized herbivores, as the populations of these species decline in the face of a changing environment.

Plant-herbivore interactions: Environmental mediation of genetic variation

Our results suggest that *A. syriaca* exhibits genetic variation for herbivore resistance, but that the local growing environment influences the expression of the genetic variation (Table 1). The maternal lines in the common garden and the maternal genets accumulated defoliation at different rates (Fig. 2), indicating genetic variation in herbivore resistance. However, defoliation of the maternal genets was uncorrelated with that of the maternal lines in the common gardens, suggesting that the location in which a milkweed clone grows determines how it responds to herbivory (G x E) (Table 1). Additionally, in the reciprocal transplant experiment, maternal lines within a transplant location accumulated defoliation at similar rates (no genetic variation), but defoliation rates differed among transplant locations (Fig. 3). In combination, these results suggest that local environment is a strong driver of defoliation. The apparent absence of genetic variation in the reciprocal transplant experiment could be explained by environmental variation changing the magnitude of response of genetic effects (Maddox & Cappuccino 1986; Maddox & Root 1987; Des Marais *et al.* 2013; Saltz *et al.* 2018), with genetic variation highly diminished in the transplant environments. Our results are in contrast with other hypotheses which suggest that expressed genetic variation should be higher in novel environments (Service & Rose 1985; Holloway *et al.* 1990; Conner *et al.* 2003). In those circumstances, we would have expected to see more genetic variation in the reciprocal transplant milkweeds in the novel transplant environments. However, we recognize that a lack of differences among maternal lines could simply reflect the small size of the reciprocal transplant milkweeds themselves (see Appendix B, Figure B4).

Genetic variation in milkweed resistance to herbivory has been extensively studied, providing evidence for genetic variation among milkweed populations in defoliation and defensive traits such as latex, cardenolides, and foliar nitrogen (Agrawal 2004b, 2005; Vannette & Hunter 2011; Agrawal & Hastings 2019a). Likewise, previous work has shown that the

population of *A. syriaca* at UMBS differs in resistance traits from other milkweed populations at regional scales (Andrews 2015). Here, we studied genetic variation in resistance traits within the UMBS population of common milkweed and associated it with phenotypic variation of maternal genets. While we observed genetic variation in some measures of resistance, our data generally support a stronger role for local environment in mediating expression of resistance. In short, among-population genetic variation may have more important effects on the expression of resistance and resistance traits than does within-population genetic variation, at least at UMBS. Additionally, the lack of correlation between the two common gardens and the maternal genets (Table 1) indicates high levels of phenotypic plasticity in this population of common milkweed, with unknown consequences for milkweed fitness under rapid environmental change. Our results suggest that genetic variation measured in a common garden does not necessarily reflect trait expression in a naturally growing population.

In other systems, evidence for genetic variation in resistance and tolerance is common in studies of herbicides (Baucom & Mauricio 2004, 2008) and herbivory (Cipollini 2002; Smith *et al.* 2008; Lehndal & Ågren 2015). At local scales, if certain maternal genets have experienced more herbivory over time than others, the effects of transgenerational resistance to herbivory (Holeski *et al.* 2012) could contribute to the lack of correlation between our maternal genets and their maternal lines. For example, wild radish, *Raphanus raphanistrum*, damaged by larvae of the cabbage white butterfly, *Pieris rapae*, are more likely to produce progeny with increased resistance (Agrawal 2002). Moreover, ontogeny is an important determinant of resistance traits (Strauss & Agrawal 1999; Muola *et al.* 2010), and the difference in ontogenetic stage between our established genets and their first year maternal lines may have influenced milkweed resistance traits. Indeed, monarch butterfly larvae consumed 26.1% of available leaf area of 4-week-old showy milkweed, *Asclepias speciosa*, while monarch larvae consumed only 5.6% of available leaf area of 12-week-old showy milkweed, indicating higher herbivore resistance in the older milkweed plants (Yang *et al.* 2020). The level of genetic variation in resistance to artificial herbivory in white swallow-wort (*Vincetoxicum hirundinaria*; Asclepiadaceae), also depends on the life-history stage of the plant (Muola *et al.* 2010). Because milkweed maternal genets in our study are at least 12 years old (M.D. Hunter, *personal communication*), it is likely that differences in age of the maternal lines and the maternal genets affects resistance to defoliation.

In addition to estimates of herbivore resistance by defoliation measures, we also measured milkweed resistance to aphid colony establishment (presence/absence) and subsequent population growth. Maternal lines did not exhibit genetic variation for resistance to *A. asclepiadis* colony establishment or population growth (Fig. 4a). Likewise, maternal lines displayed no genetic variation in resistance to *M. asclepiadis* (Fig. 4b), although maternal genets varied in resistance to *M. asclepiadis* colony growth (Fig. 5). These results suggest that genetic variation within our population does not influence *A. asclepiadis* dynamics on common milkweed, whereas local growing environment appears to mediate the resistance of common milkweed to *M. asclepiadis*. However, genetic variation within common milkweed populations influences variation in *A. asclepiadis* abundance 5.5-fold under conditions of interspecific competition among aphids (Smith *et al.* 2008). Beyond milkweed, among-population genetic variation in *Oenothera biennis*, evening primrose, accounted for 19.62% of arthropod abundance and 11.01% of arthropod species richness (Johnson & Agrawal 2005). Although our results do not support the hypothesis that genetic variation at local scales influences aphid population dynamics, it is possible that a longer-term experiment with a larger source of genetic variation would uncover stronger effects of genotype.

Plant foliar quality: Variations in sources of variation

Genotype-by-environment interactions in plant chemistry are well-studied (Vannette & Hunter 2011; Des Marais *et al.* 2013; Aslam & Karanja 2020), although their relative and interactive contributions to standing phenotypic variation in natural populations are rarely known. Here, we can assess drivers of variation in milkweed foliar quality, including chemical defense. We detected no genetic variation in foliar cardenolide concentrations among maternal lines (Fig. 6a, b) but did observe variation in cardenolide concentration among naturally growing maternal genets (Fig 6c). Foliar cardenolide concentrations from maternal lines were three- to four-fold higher in the greenhouse than in the field common garden, (Fig. 6a, b), amply demonstrating how growing environment can influence the expression of resistance traits. We detected no differences in cardenolide concentrations among reciprocal transplant maternal lines or transplant locations (Fig. 7), but small plants and limited data likely inhibit accurate conclusions from these results.

Overall, our results from the common gardens and maternal genets indicate that variation in cardenolide concentrations are substantially influenced by the environment in which milkweeds grow. Indeed, many milkweed species induce cardenolide defense with herbivory (Malcolm & Zalucki 1996; Rasmann *et al.* 2009; Rasmann & Agrawal 2011, but see Zehnder & Hunter 2007), and therefore herbivory may be responsible for the variation in cardenolide concentrations that we observed in the maternal genets. However, cardenolides are also known to vary genetically (Agrawal 2005). Theory suggests that inducible defenses trade off with constitutive defenses (Zangerl & Bazzaz 1992; Rasmann *et al.* 2011) but they can also correlate positively (Rasmann & Agrawal 2011). Accordingly, variation in foliar cardenolides among milkweeds could represent combinations of both constitutive and inducible cardenolide concentrations. Defoliation from chewing herbivores and cardenolide concentration were highly positively correlated in the maternal genets (Appendix A, Table A2), strongly suggesting that herbivores are inducing cardenolides in the maternal genets. Additionally, maternal genets may be expressing higher levels of cardenolides due to priming of chemical defense against herbivory over multiple years (Frost *et al.* 2008). In contrast, cardenolides and defoliation were uncorrelated in the maternal lines of the field common garden, further demonstrating phenotypic plasticity in cardenolide expression in response to the local environment. Cardenolides are induced by aboveground herbivores but also root herbivores such as the larvae of the red milkweed beetle, *Tetraopes tetraophthalmus,* (Rasmann *et al.* 2011) and interactions with mycorrhizal fungi (Meier & Hunter 2018). Thus, the data that we collected from field plants likely includes responses to drivers of foliar chemistry that we neither measured nor controlled during our study.

Our results suggest that variation in foliar nitrogen in this study is also driven largely by the environment. We observed no genetic variation in foliar nitrogen concentrations among maternal lines (Fig. 8a, b) but did observe differences among the maternal genets in foliar nitrogen (Fig. 8c), indicating that local growing conditions may determine foliar nitrogen concentrations. Previous common garden studies identify among-population genetic variation in foliar nitrogen of common milkweed maternal lines (Agrawal 2004b, 2005), but lack of genetic variation in nitrogen has been identified in other plant species such as aspen (Rytter & Stener 2003). However, interactions with environmental conditions are likely to invoke variability: both above- and below-ground herbivore attack cause milkweed to preferentially allocate nitrogen

away from sites of damage and into stems (Tao & Hunter 2013), but we observed no correlation between foliar C:N ratios and defoliation (Appendix A, Table A2). Therefore, unrecorded below-ground herbivory could have contributed to patterns observed in the maternal genets and to the lack of correlation between defoliation and foliar C:N ratios.

In contrast to nitrogen, we did observe genetic variation in foliar carbon concentrations: maternal lines in both of the common gardens expressed genetic variation (Fig. 9a, b), while the maternal genets also varied in foliar carbon (Fig. 9c). The foliar carbon concentrations of maternal lines in both common gardens were correlated with those of the maternal genets, yet the two common gardens were uncorrelated with each other. Although this result is puzzling, it indicates that plants grown in the greenhouse express traits very differently than those in the field (Cipollini 2002; Conner *et al.* 2003; Forero *et al.* 2019), demonstrating the broad phenotypic plasticity of common milkweed.

Our final measure of plant foliar quality and defense was foliar latex. Foliar latex is known to vary among milkweed genotypes both within- and among-populations (Agrawal 2005; Agrawal & Hastings 2019a), and we observed genetic variation in latex exudation in both common gardens and observed variation among the maternal genets (Fig. 10). Nevertheless, the latex exudation of the maternal lines remained uncorrelated between the field common garden and the greenhouse common garden and between the maternal lines of both common gardens and the maternal genets. This result again indicates that although genetic variation plays a role in determining foliar latex exudation, local environmental growing conditions dictate the level and pattern of that expression, i.e. phenotypic plasticity. Latex exudation of maternal lines in the field common garden was negatively genetically correlated with defoliation (Appendix A, Table A2), supporting that latex is an effective defense against chewing herbivores. It also suggests that, if defoliation reduces milkweed fitness, we should expect directional selection for increased latex exudation over time. In contrast, defoliation and latex exudation were uncorrelated in the maternal genets (Appendix A, Table A2). However, latex production that is induced two-fold by monarch caterpillar herbivory can be returned to pre-monarch levels by simultaneous root herbivory (Rasmann *et al.* 2009). This result reminds us that unrecorded below-ground herbivory may confound correlations among resistance traits and herbivore activity.

Conclusions

Our results suggest that underlying sources of milkweed phenotypic diversity are complex and vary among traits. This study allowed us to measure within-population genetic variation in resistance traits of common milkweed and then compare those traits with patterns of resistance in the naturally growing milkweed population. We could therefore assess how local growing environment dictates trait expression. We identified one type of milkweed defense, foliar cardenolides, as predominantly environmentally controlled, while another, foliar latex, displayed significant genetic variation. Additionally, some milkweed-aphid interactions were explained best by local environment while others remain largely unexplained. Milkweed resistance to chewing herbivores was genetically variable but mediated by the local growing environment. Similar to the findings of many other studies, plant phenotypic variation in our milkweed population is due to the interactions between genetics and the environment (Via & Lande 1985; Hahn *et al.* 2019; Aslam & Karanja 2020) and this interaction mediates plant-herbivore interactions (Maddox & Cappuccino 1986; Johnson & Agrawal 2005; Vannette & Hunter 2011). The results of our study indicate which milkweed traits may be acted upon locally by natural selection (Via & Lande 1985), and may therefore be most likely to adapt to ongoing environmental change. Our results suggest that, even if genetic variation exists within a population, phenotypic variation may be determined mainly by the local growing environment. Because of this, it is unclear if local populations have the ability to adapt to rapid environmental change: local population adaptation to rapid environmental change may require dispersal or migration of novel genotypes. However, our study lacks information on below-ground herbivory, plant ontogeny, regional trait variation and fine scale chemical analysis. Future research that addresses these shortcomings and records similar data over time and among regional populations is necessary to understand the mechanisms behind the patterns we observed in our study.

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Author contributions

A.S.P. and M.D.H. designed the experiments. A.S.P. and L.R. collected the data. A.S.P. conducted cardenolide analyses, T.V. conducted C:N analyses. A.S.P. and M.D.H. analyzed the data. A.S.P. wrote the manuscript, and M.D.H. contributed to drafts.

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Tables & Figures

Trait	1 Field CG	2 Greenhouse CG	3 Field CG = Greenhouse	4 Maternal Genets	5 Field CG = Maternals	6 Greenhouse = Maternals
Resistance (defoliation)	YES			YES	NO	
Resistance (*A. asclepiadis*)	NO					
Resistance (*M. asclepiadis*)	NO			YES	NO	
Latex Exudation	YES	YES	NO	YES	NO	NO
Cardenolide Concentration	NO	NO	NO	YES	NO	NO
Foliar Nitrogen	NO	NO	NO	YES	NO	NO
Foliar Carbon	YES	YES	NO	YES	YES	YES
Height	YES	YES	NO	YES	NO	NO
Leaf Number	YES	YES	YES	YES	NO	NO
Stem Diameter	YES	YES	NO	YES	NO	NO

Table 1 - Summary of analyses exploring variation among maternal lines (in the field and greenhouse common gardens) and maternal genets in their resistance and growth traits. "YES" indicates evidence of significant variation among maternal lines or maternal genets (columns 1, 2, 4) or of significant genetic correlations in those traits between the common gardens and the maternal genets (columns 3, 5, 6). "CG" refers to common garden, whereas "Maternal" refers to the natural population of maternal genets. We observed (a) pervasive variation among maternal genets in their resistance and growth traits (column 4), but (b) a general lack of correlations among those traits between the common gardens (column 3) or between trait variation in either common garden and the variation among maternal genets (columns 5, 6).

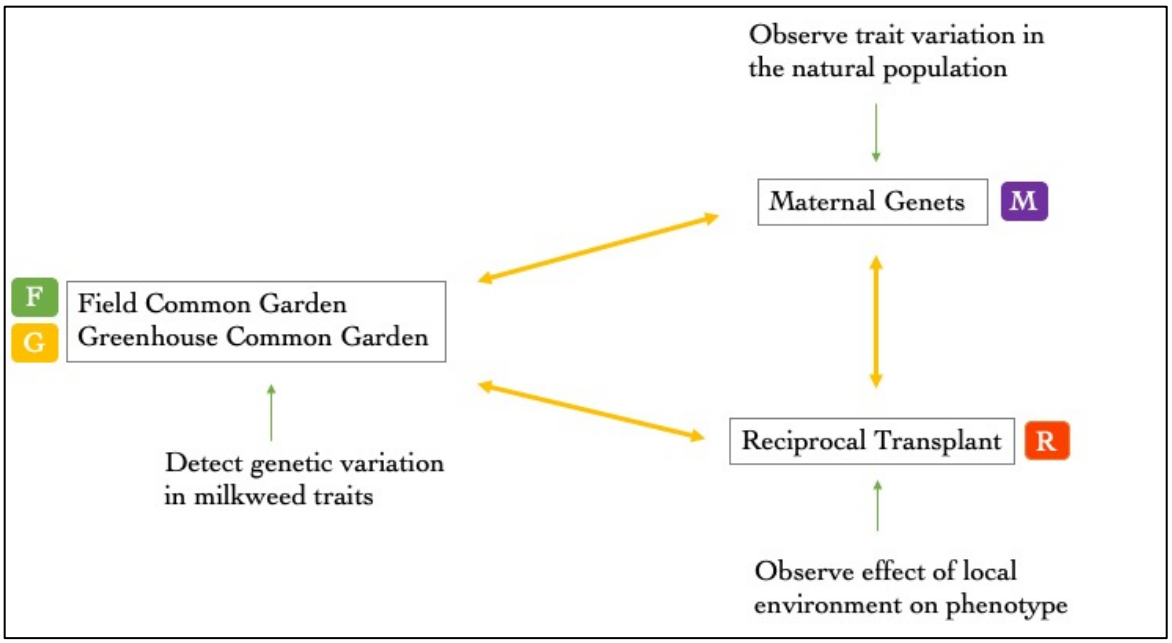

Figure 1a - Concept diagram of an experimental set up consisting of a field and a greenhouse common garden, a reciprocal transplant experiment, and sampling of naturally growing milkweed maternal genets. The diagram also explains the primary goal of each experimental group.

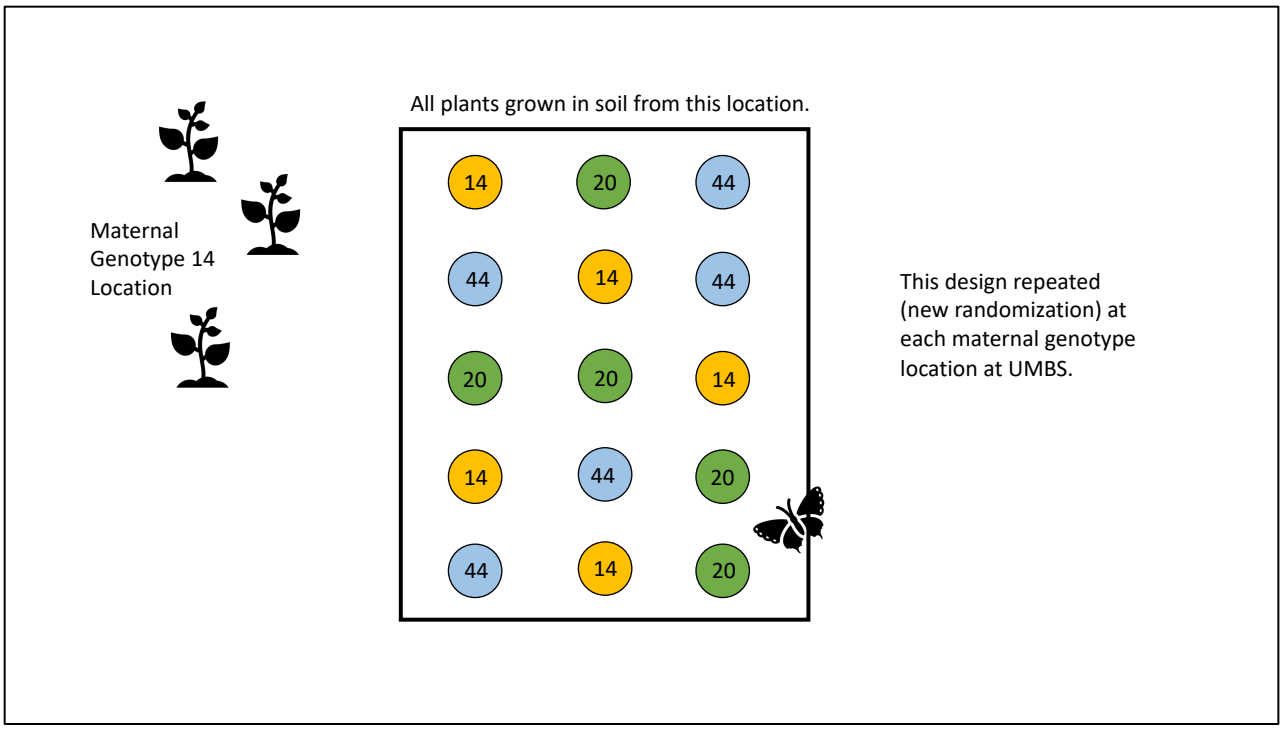

Figure 1b - Experimental set up of a reciprocal transplant experiment of common milkweed. Replicate seedlings of three maternal lines (14, 20 & 44) were planted at each of 3 locations, with each location representing the maternal genet of one of the maternal lines. Only one location (maternal genet) is illustrated here.

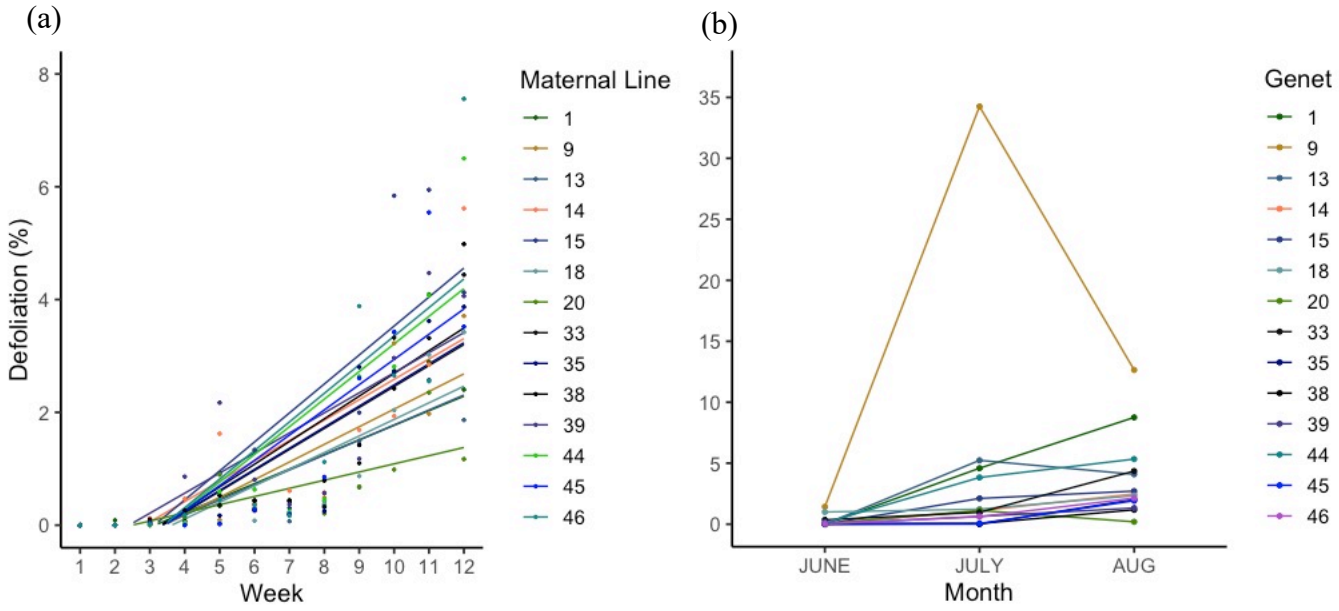

Figure 2 – Percent defoliation of milkweeds in (a) a field common garden and (b) their unmanipulated maternal genets. Field common garden maternal lines expressed genetic variation in rate of resistance to herbivory (Week*Maternal line, $F_{13, 2746} = 2.30$, $P = 0.0051$). Maternal genets displayed variation in herbivory resistance (Maternal genet*Month, $F_{26, 110} = 6.56$, $P < 0.0001$). Points represent the mean defoliation for the maternal line/genet for the week/month. Points represent mean defoliation of (a) 18 milkweeds per maternal line, and (b) 5 milkweeds per maternal genet. Lines in (a) are regressions, while lines in (b) are for visual reference only. High July defoliation in maternal genet 9 was due to extensive deer browsing. Note difference in y-axis scale.

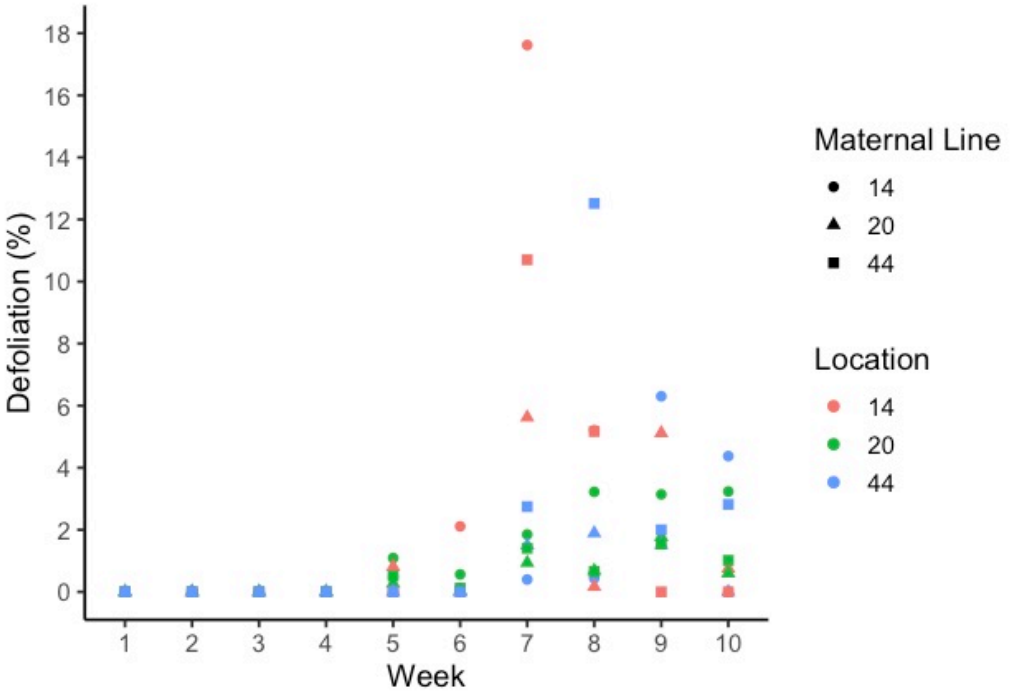

Figure 3– Percent defoliation of milkweeds in a reciprocal transplant experiment. Percent defoliation did not differ among maternal lines ($F_{2, 36} = 0.27$, P = 0.7668) but did differ among maternal transplant locations ($F_{2, 36} = 3.44$, P = 0.0428; Week*Location $F_{2, 392} = 22.53$, P <0.0001). Maternal lines did not express variation in resistance to herbivory based on their location (Maternal line*Location, $F_{4, 36} = 0.20$, P = 0.9381). Points represent mean defoliation of 5 milkweeds of the maternal line at a location.

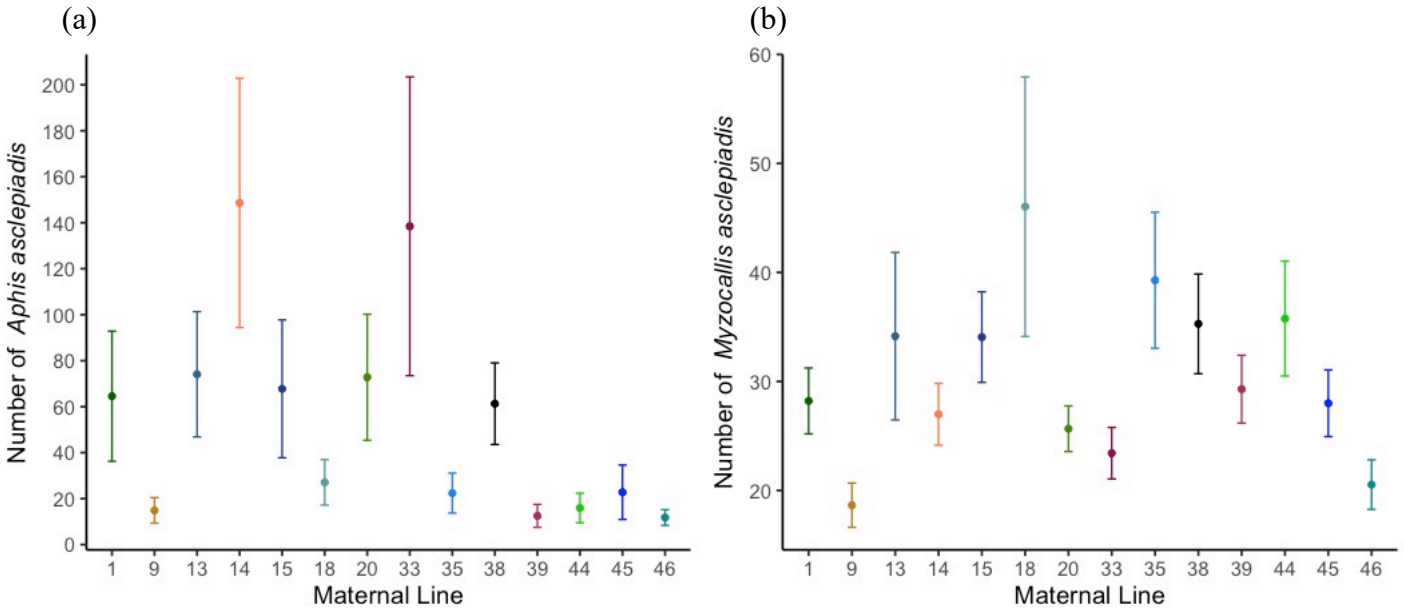

Figure 4 – Mean population sizes of (a) *Aphis asclepiadis* and (b) *Myzocallis asclepiadis* in a field common garden of milkweed maternal lines. Data are for milkweeds on which *A. asclepiadis* and *M. asclepiadis* were present. Maternal lines did not show genetic variation for resistance to *A. asclepiadis* population growth ($F_{13, 111} = 1.08$, P = 0.3859; Week*Maternal line, $F_{13, 130} = 1.23$, P = 0.2683) or *M. asclepiadis* population growth ($F_{13, 219} = 1.22$, P = 0.2645; Week*Maternal line, $F_{13, 910} = 1.49$, P = 0.1133). Points are means of (a) 287 samples (*A. asclepiadis*) and (b) 1172 samples (*M. asclepiadis*), and error bars are ± 1 SE. Data were log transformed before statistical analysis. Note difference in y-axis scale.

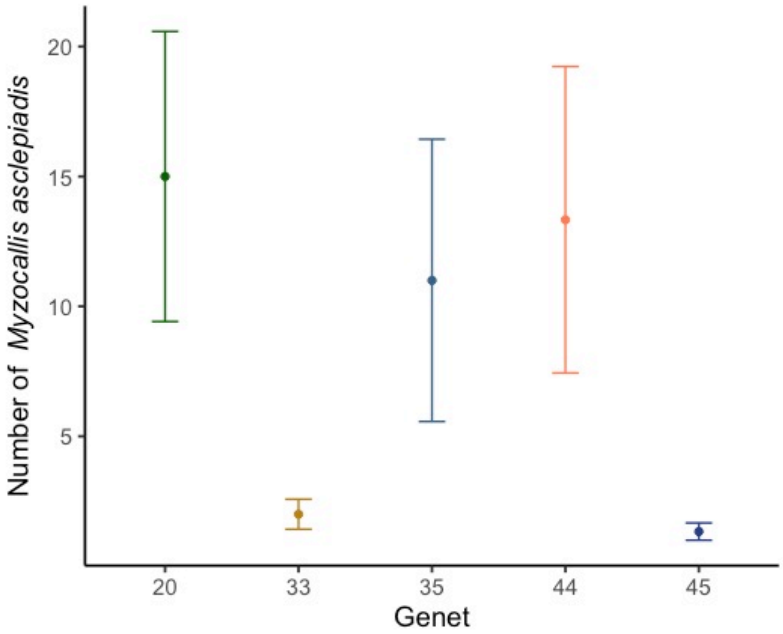

Figure 5 – Mean population sizes of *Myzocallis asclepiadis* on unmanipulated maternal milkweed genets. Data are for milkweeds on which *M. asclepiadis* were present. *M. asclepiadis* population sizes differed among maternal genets ($F_4 = 5.50$, $P = 0.0095$). Points are means of 17 samples (*M. asclepiadis*), and error bars are ± 1 SE. Data were log transformed before statistical analysis.

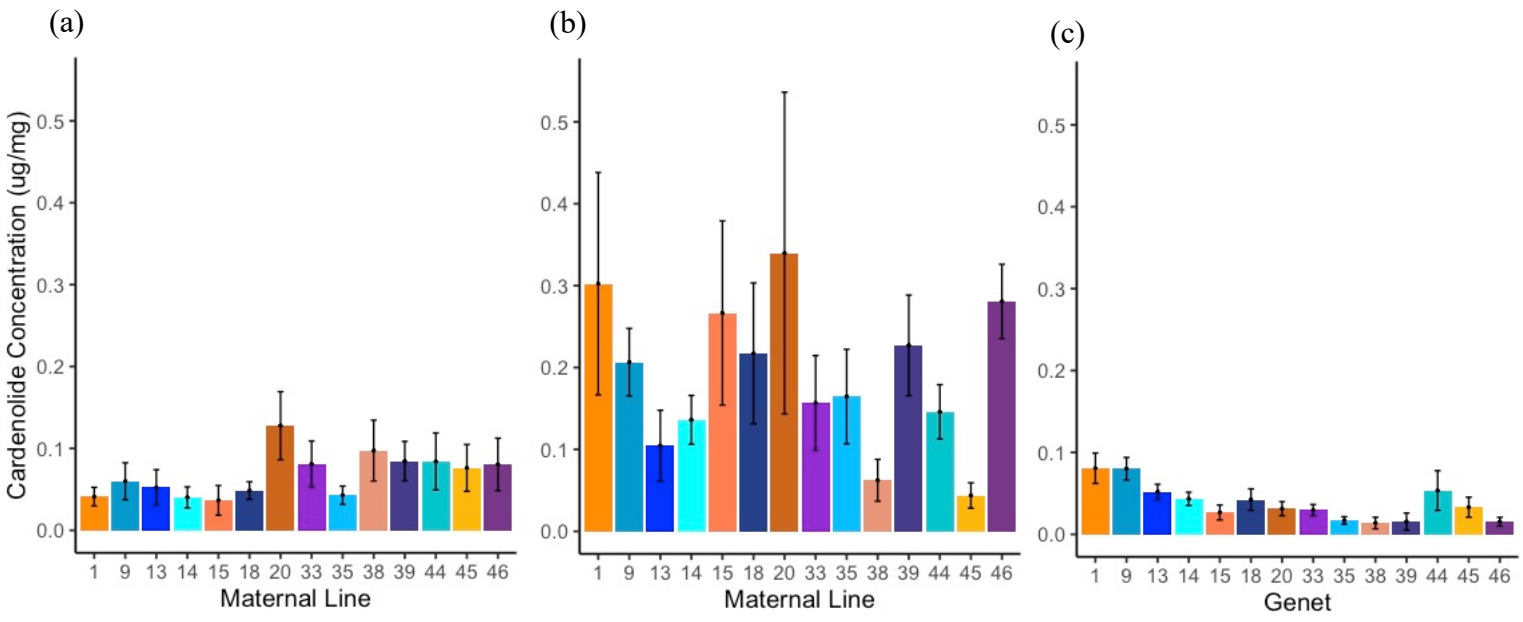

Figure 6 – Foliar cardenolide concentrations of milkweed maternal lines in a (a) field common garden, (b) greenhouse common garden, and (c) their maternal genets. Neither the field common garden nor the greenhouse common garden milkweeds displayed genetic variation in cardenolide concentration ($F_{13, 101} = 1.11$, $P = 0.3568$; $F_{13, 98} = 1.53$, $P = 0.1212$, respectively). The maternal genets varied in cardenolide concentration ($F_{13} = 3.42$, $P = 0.0007$). Data were log-transformed prior to analysis. Bars represent (a, b) 9 milkweeds per maternal line and (c) 5 milkweeds per maternal genet. Error bars are ± 1 SE.

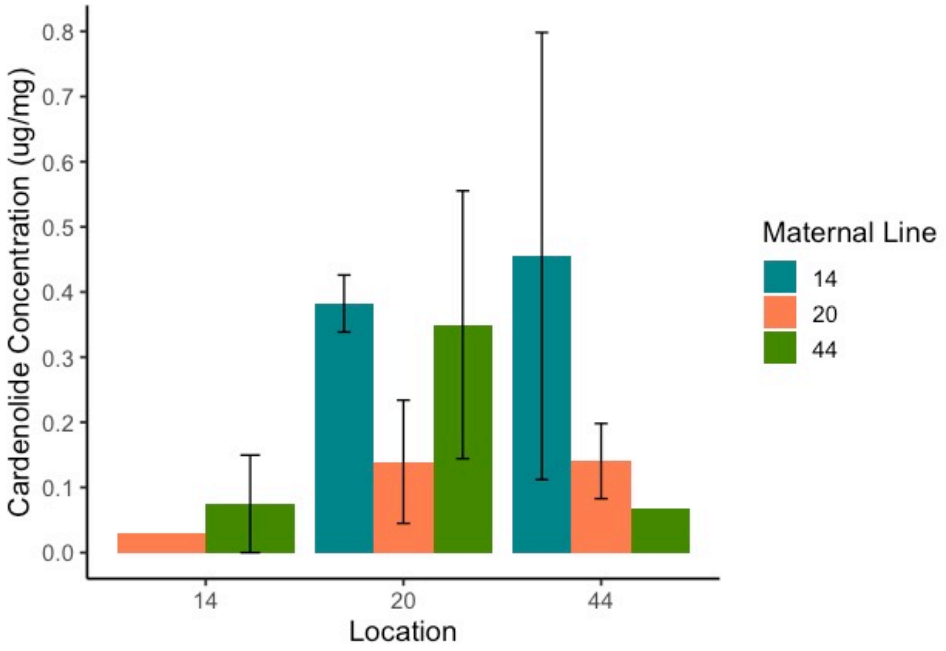

Figure 7 – Foliar cardenolide concentrations of maternal lines in a reciprocal transplant experiment. Foliar cardenolide concentrations in milkweed did not differ among milkweed maternal lines ($F_2 = 1.20$, $P = 0.3316$) or maternal transplant locations ($F_2 = 0.44$, $P = 0.6552$). The location in which a maternal line was grown did not affect cardenolide concentration (Maternal line*Location, $F_3 = 0.18$, $P = 0.9096$). Data were log-transformed prior to analysis. Maternal line 14 is not represented in Location 14 because not enough leaf tissue remained for chemistry samples after heavy herbivory. Error bars are ± 1 SE.

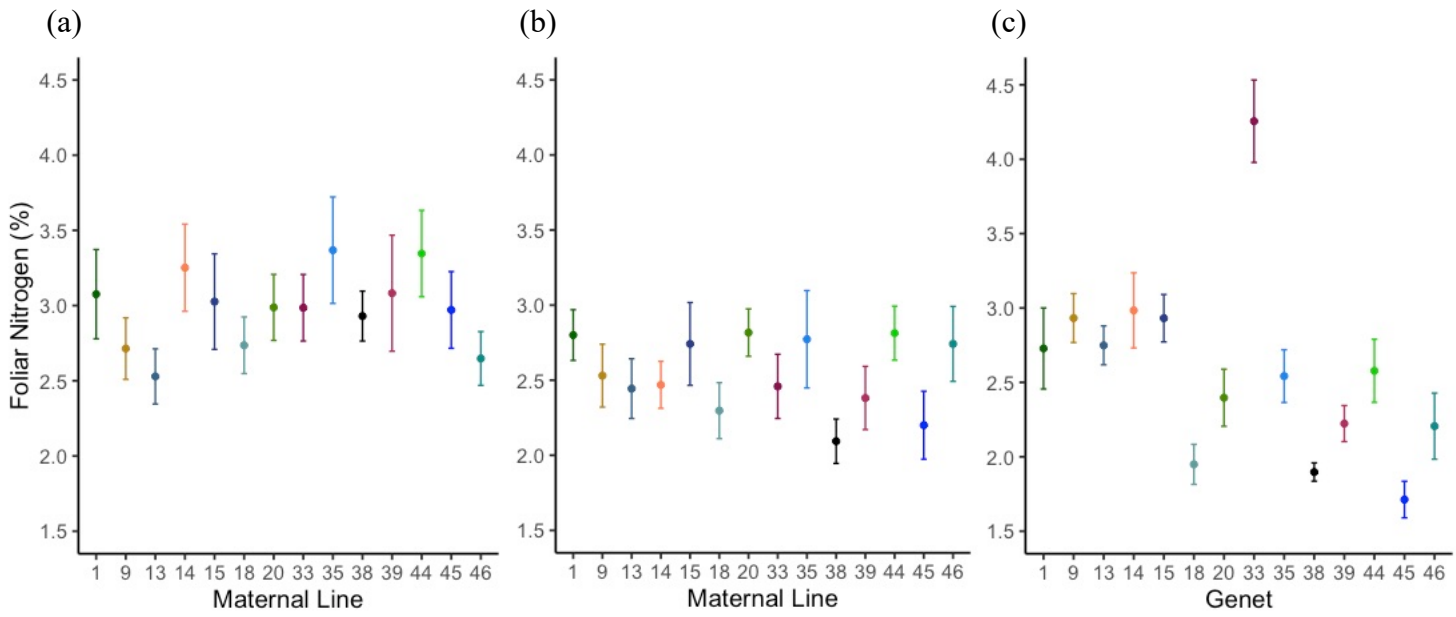

Figure 8 – Mean percent foliar nitrogen concentration of maternal lines in a (a) field common garden, (b) greenhouse common garden, and (c) their maternal genets. Neither the field nor greenhouse common garden milkweeds displayed genetic variation in percent nitrogen ($F_{13, 104} = 1.38$, $P = 0.1821$; $F_{13, 104} = 1.73$, $P = 0.0659$, respectively). The maternal genets varied in percent nitrogen ($F_{13, 56} = 11.21$, $P < 0.0001$). Points are means of (a, b) 9 milkweeds per maternal line and (c) 5 milkweeds per maternal genet. Error bars are ± 1 SE.

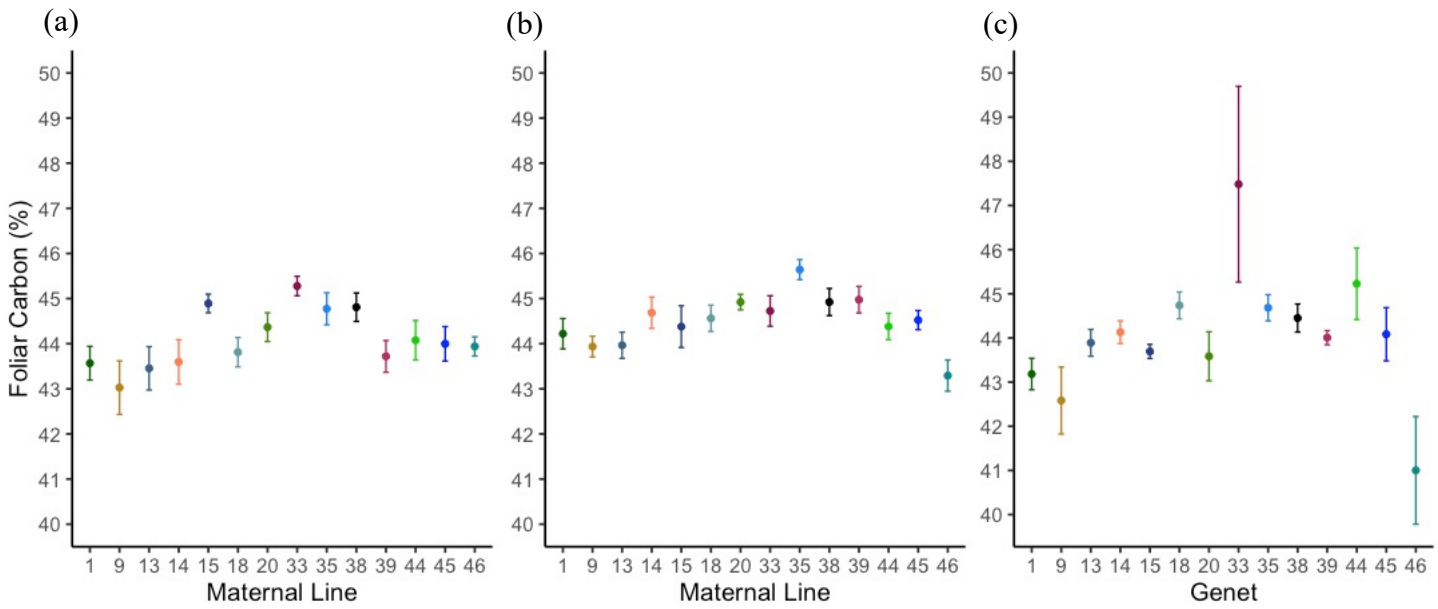

Figure 9 – Mean percent foliar carbon concentration of maternal lines in a (a) field common garden, (b) greenhouse common garden, and (c) their maternal genets. The milkweed in the field and greenhouse common gardens displayed genetic variation in percent carbon ($F_{13, 104} = 3.06$, $P = 0.0007$; $F_{13, 104} = 3.46$, $P = 0.0002$). The maternal genets also varied in percent foliar carbon ($F_{13, 56} = 3.23$, $P = 0.0011$). Points are means of (a, b) 9 milkweeds per maternal line and (c) 5 milkweeds per maternal genet. Error bars are ± 1 SE.

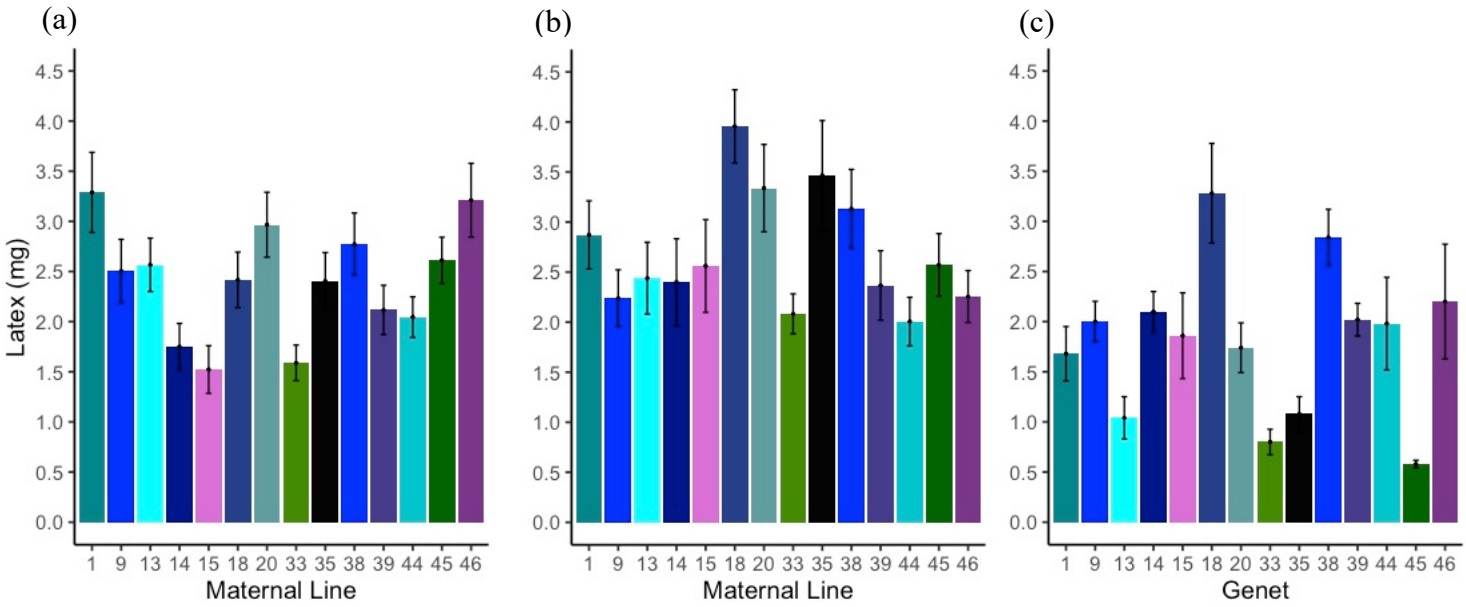

Figure 10 – Mean foliar latex exudation of maternal lines in a (a) field common garden, (b) greenhouse common garden, and (c) their maternal genets. Maternal lines displayed genetic variation in foliar latex exudation in the field and greenhouse common gardens ($F_{13, 221} = 3.89$, $P < 0.0001$; $F_{13, 221} = 2.49$, $P = 0.0034$, respectively), and foliar latex exudation varied among the maternal genets ($F_{13} = 5.64$, $P < 0.0001$). Bars represent (a, b) 18 milkweeds per maternal line and (c) 5 milkweeds per maternal genet. Error bars are ± 1 SE.

Appendix A

Group	Arthropod	June	July	August
Asclepias specialist	*Danaus plexippus* larva	15	41	16
Asclepias specialist	*Danaus plexippus* egg	13	84	3
Asclepias specialist	*Rhyssomatus lineaticollis*	4	5	7
Asclepias specialist	*Tetraopes tetrophthalmus*	0	0	10
Asclepias specialist	*Aphis asclepiadis*	541	15977	1604
Asclepias specialist	*Myzocallis asclepiadis*	15	10170	25744
Asclepias specialist	*Liriomyza asclepiadis*	5	514	522
Asclepias specialist	*Lygeus kalmii*	2	45	6
Asclepias specialist	*Euchaetes egle* larva	0	607	134
Predator	Spider (unident.)	99	230	195
Predator	Coccinelid	1	22	35
Predator	Mirid bug	0	14	4
Predator	Lacewing larva	0	4	2
Predator	Syrphid fly larva	0	6	2
	TOTAL	695	27719	28284

Table A1 – Total arthropods counted on milkweed maternal lines in the field common garden in June, July, and August. Arthropods were identified to level listed above.

Location	Variable X	Variable Y	Equation	R-squared	P-value
Field Common Garden	Growth PCA	Cardenolides	y = 0.0093x + 0.0681	R^2 = 0.2348	P = 0.0791
Greenhouse Common Garden	Growth PCA	Cardenolides	y = 0.0041x + 0.1896	R^2 = 0.0051	P = 0.8084
Maternal Genets	Growth PCA	Cardenolides	y = 0.0054x + 0.0382	R^2 = 0.1013	P = 0.2674
Field Common Garden	Growth PCA	Defoliation	y = -0.0115x + 0.4447	R^2 = 0.0026	P = 0.8613
Maternal Genets	Growth PCA	Defoliation	y = 3.1675x + 3.9864	R^2 = 0.2171	P = 0.0931
Field Common Garden	Growth PCA	Latex	y = 9E-05x + 0.0024	R^2 = 0.0576	P = 0.4087
Greenhouse Common Garden	Growth PCA	Latex	y = -0.0002x + 0.0029	R^2 = 0.1455	P = 0.1784
Maternal Genets	Growth PCA	Latex	y = -0.0002x + 0.0018	R^2 = 0.0846	P = 0.3130
Field Common Garden	Growth PCA	C:N	y = 0.434x + 14.888	R^2 = 0.2460	P = 0.0713
Greenhouse Common Garden	Growth PCA	C:N	y = -0.3684x + 17.683	R^2 = 0.0971	P = 0.2782
Maternal Genets	Growth PCA	C:N	y = -2.1629x + 17.927	R^2 = 0.5045	**P = 0.0044**
Field Common Garden	Cardenolides	Defoliation	y = -2.6695x + 0.6752	R^2 = 0.1653	P = 0.7168
Maternal Genets	Cardenolides	Defoliation	y = 208.58x - 5.2871	R^2 = 0.8878	**P = 0.0094**
Field Common Garden	Latex	Defoliation	y = -295.3x + 1.1569	R^2 = 0.2826	**P = 0.0504**
Maternal Genets	Latex	Defoliation	y = 636.13x + 2.8414	R^2 = 0.0029	P = 0.8558
Field Common Garden	C:N	Defoliation	y = -0.0274x + 0.8756	R^2 = 0.0100	P = 0.7335
Maternal Genets	C:N	Defoliation	y = -0.7372x + 17.474	R^2 = 0.1100	P = 0.2466
Field Common Garden	Cardenolides	Latex	y = 0.0072x + 0.002	R^2 = 0.1497	P = 0.1717
Greenhouse Common Garden	Cardenolides	Latex	y = 0.0004x + 0.0028	R^2 = 0.0033	P = 0.8451
Maternal Genets	Cardenolides	Latex	y = -0.001x + 0.0018	R^2 = 0.0009	P = 0.9200
Field Common Garden	C:N	Latex	y = 0.0001x + 0.0006	R^2 = 0.0915	P = 0.2933
Greenhouse Common Garden	C:N	Latex	y = 0.0001x + 0.0006	R^2 = 0.1222	P = 0.2205
Maternal Genets	C:N	Latex	y = 5E-05x + 0.0008	R^2 = 0.0838	P = 0.3153
Field Common Garden	C:N	Cardenolides	y = 0.0011x + 0.0523	R^2 = 0.0024	P = 0.8692
Greenhouse Common Garden	C:N	Cardenolides	y = -0.0341x + 0.7928	R^2 = 0.4891	**P = 0.0054**
Maternal Genets	C:N	Cardenolides	y = -0.0019x + 0.0724	R^2 = 0.1173	P = 0.2307

Table A2 – Correlations between milkweed resistance and growth traits within each common garden and within the maternal genets. Significant results are boldface.

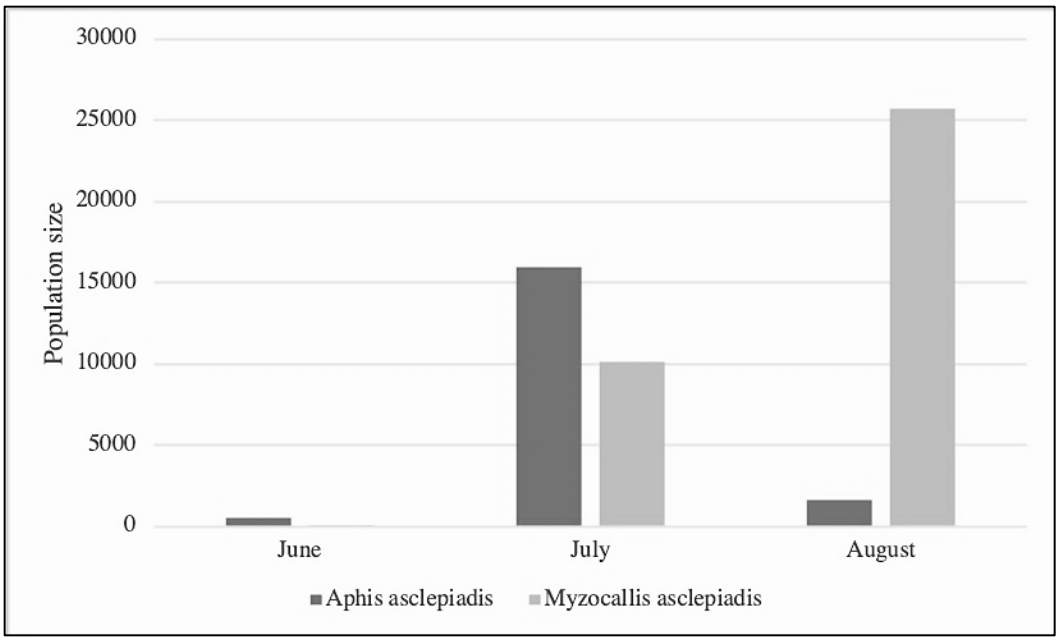

Figure A1 – Population sizes of *Aphis asclepiadis* and *Myzocallis asclepiadis* in the field common garden. Almost all insects recorded in August were *Myzocallis asclepiadis*.

Appendix B

Plant Growth

The maternal lines grown in both the field and greenhouse common garden expressed genetic variation in leaf production and senescence rate (Week*Week*Maternal line, $F_{14, 2733} = 25.18$, $P < 0.0001$; Week*Week*Maternal line, $F_{14,2744} = 35.66$, $P < 0.0001$, respectively, Figure B1a, b). Initial rates of leaf growth were correlated between the two gardens ($y = 1.8967x + 0.4142$, $R^2 = 0.8383$, $P < 0.0001$). Maternal genets also varied in leaf production rates (Maternal line*Month, $F_{26, 110} = 2.71$, $P = 0.0001$), but maternal genet leaf production was uncorrelated with leaf production in either common garden (Field, $y = 0.8186x + 0.9316$, $R^2 = 0.0254$, $P = 0.5865$; Greenhouse, $y = 0.352x + 0.8101$, $R^2 = 0.0717$, $P = 0.3546$). In contrast to the common gardens, maternal lines in the reciprocal transplant experiment had similar rates of leaf production (Week*Maternal line, $F_{2, 392} = 1.45$, $P = 0.2358$; Maternal line*Location, $F_{4, 36} = 0.20$, $P = 0.9381$, Figure B2). Notably, leaf production rates varied among transplant locations (Week*Location, $F_{2, 392} = 22.53$, $P < 0.0001$, Figure B2), indicating the importance of local resources for milkweed growth rates.

Maternal lines in both the field and greenhouse common garden displayed genetic variation in height growth and senescence rate (Week*Week*Maternal line, $F_{14, 2733} = 2.83$, $P = 0.0003$; Week*Week*Maternal line, $F_{14, 2744} = 10.99$, $P < 0.0001$, Figure B3a, b). However, the initial height growth rates of the common gardens were uncorrelated ($y = 0.0831x + 0.7929$, $R^2 = 0.0005$, $P = 0.9425$). Maternal genets varied in height ($F_{13, 56} = 6.43$, $P < 0.0001$) and height growth rate (Maternal line*Month, $F_{26, 110} = 2.71$, $P = 0.0002$), but initial height growth rates of the maternal genets were not predicted by the field or the greenhouse common gardens ($y = 0.8186x + 0.9316$, $R^2 = 0.0254$, $P = 0.5865$; $y = 0.352x + 0.8101$, $R^2 = 0.0717$, $P = 0.3546$, respectively).

Plant heights in the reciprocal transplant experiment did not vary among maternal lines or maternal transplant locations (Week*Maternal line, $F_{2, 395} = 2.88$, $P = 0.0576$; Location, $F_{2, 36} = 1.25$, $P = 0.2983$; Maternal line*Location, $F_{4, 36} = 0.09$, $P = 0.9858$, Figure B4), and height did not vary among transplant locations (Week*Location, $F_{2, 395} = 1.46$, $P = 0.2334$, Figure B4). It is possible that plants were still too small for differences to emerge by the end of the growing season.

Maternal lines in both the field and greenhouse common gardens expressed genetic variation in base stem diameter growth and senescence rates (Week*Week*Maternal line, $F_{14, 2733} = 23.29$, $P < 0.0001$; Week*Week*Maternal line, $F_{14, 2744} = 16.33$, $P < 0.0001$, respectively, Figure B5a, b), but were uncorrelated between gardens ($y = -0.217x + 0.2535$, $R^2 = 0.0158$, $P = 0.6689$). Maternal genets also varied in base stem diameter ($F_{13, 56} = 4.54$, $P < 0.0001$) but not base stem diameter growth rates (Maternal line*Month, $F_{26, 110} = 1.34$, $P = 0.1495$). Neither the field common garden nor the greenhouse common garden stem diameter initial growth rates were correlated with those of the maternal genets ($y = -0.0565x + 0.11$, $R^2 = 0.002$, $P = 0.8797$; $y = 0.1464x + 0.0693$, $R^2 = 0.0399$, $P = 0.4933$, respectively).

Appendix B – Figures

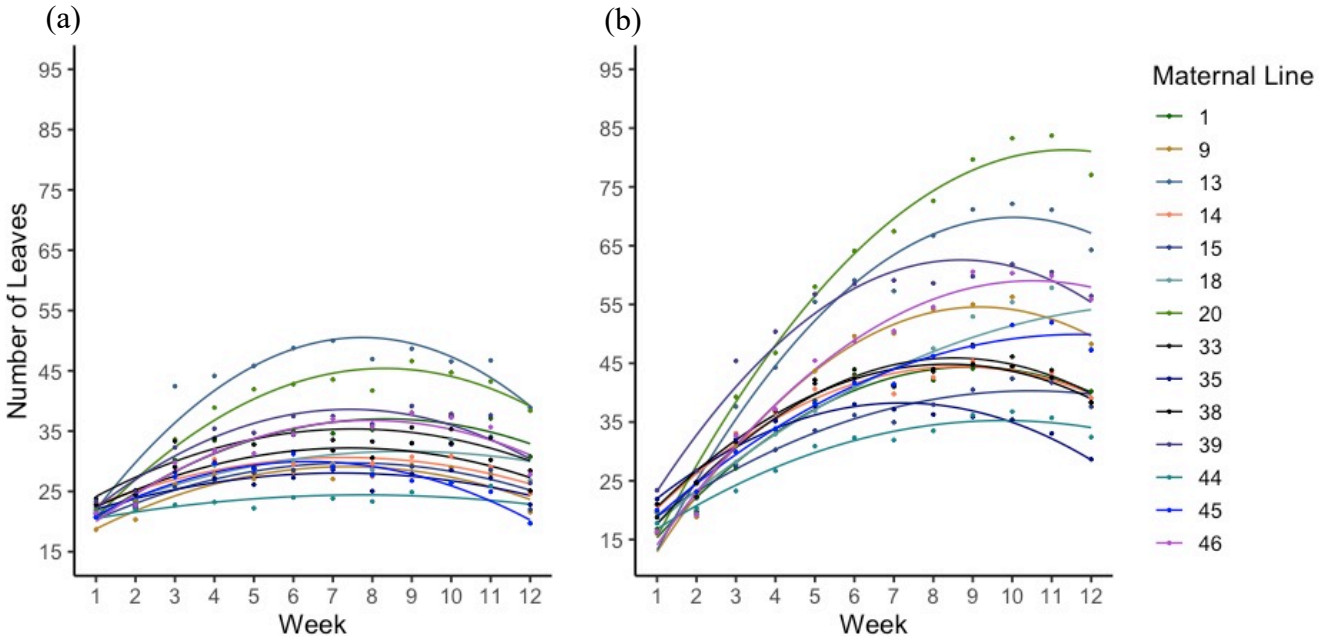

Figure B1 – Mean leaf production throughout the season of milkweed maternal lines in (a) a field common garden and (b) a greenhouse common garden. The maternal lines in both the field common garden and greenhouse common displayed genetic variation in rate of leaf production and senescence (Week*Week*Maternal line $F_{14, 2733} = 25.18$, $P < 0.0001$; Week*Week*Maternal line $F_{14,2744} = 35.66$, $P < .0001$, respectively). Points represent the mean height of 18 milkweeds of a maternal line for each week.

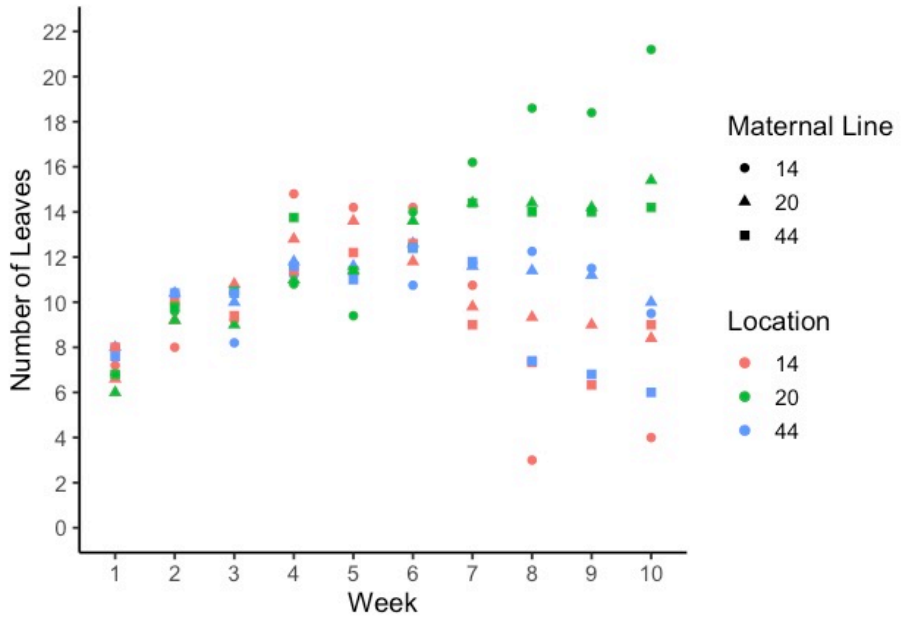

Figure B2 – Mean leaf production of milkweed maternal lines in a reciprocal transplant experiment. Leaf production did not vary among maternal lines ($F_{2, 36} = 0.27$, $P = 0.7668$), but did vary among maternal transplant locations ($F_{2, 36} = 3.44$, $P = 0.0428$, Week*Location, $F_{2, 392} = 22.53$, $P < 0.0001$). Maternal lines did not produce different numbers of leaves depending on their location (Maternal line*Location, $F_{4, 36} = 0.20$, $P = 0.9381$). Points represent mean leaf number of 5 milkweeds of a maternal line in a location for the given week.

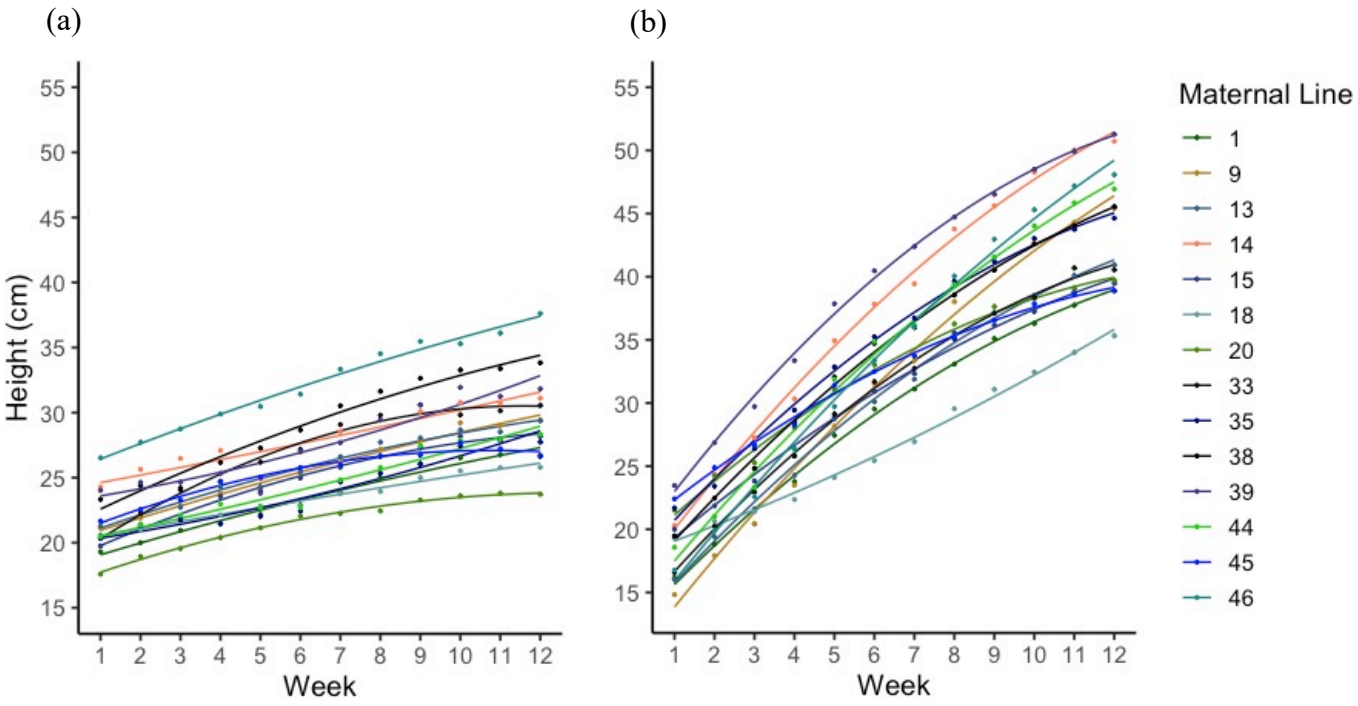

Figure B3 – Mean height (cm) of milkweed maternal lines throughout the season in a (a) field common garden and a (b) greenhouse common garden. Maternal lines in the field and greenhouse common gardens displayed genetic variation in height growth rate and senescence rate (Week*Week*Maternal line, $F_{14, 2733} = 2.83$, $P = 0.0003$; Week*Week*Maternal line, $F_{14, 2744} = 10.99$, $P < 0.0001$, respectively). Points represent the mean height of 18 milkweeds of a maternal line for each week.

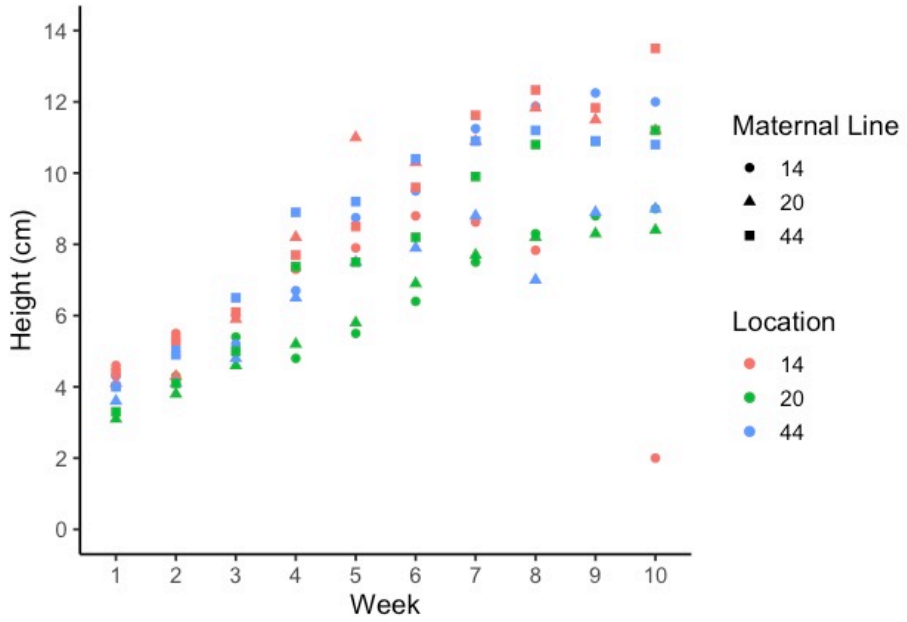

Figure B4 – Height (cm) of milkweeds in a reciprocal transplant experiment. Milkweed height did not differ among maternal lines ($F_{2, 36} = 0.39$, P = 0.6773) and did not differ among maternal transplant locations ($F_{2, 36} = 1.25$, P = 0.2983, Week*Location, $F_{2, 395} = 1.46$, P = 0.2334). Maternal lines did not grow to different heights based on their maternal transplant location (Maternal line*Location, $F_{4, 36} = 0.09$, P = 0.9858). It may be possible that plants were small enough throughout the sampling period that differences in height did not emerge. Points represent mean height of 5 milkweeds of the maternal line in a location for the given week, 45 milkweeds total.

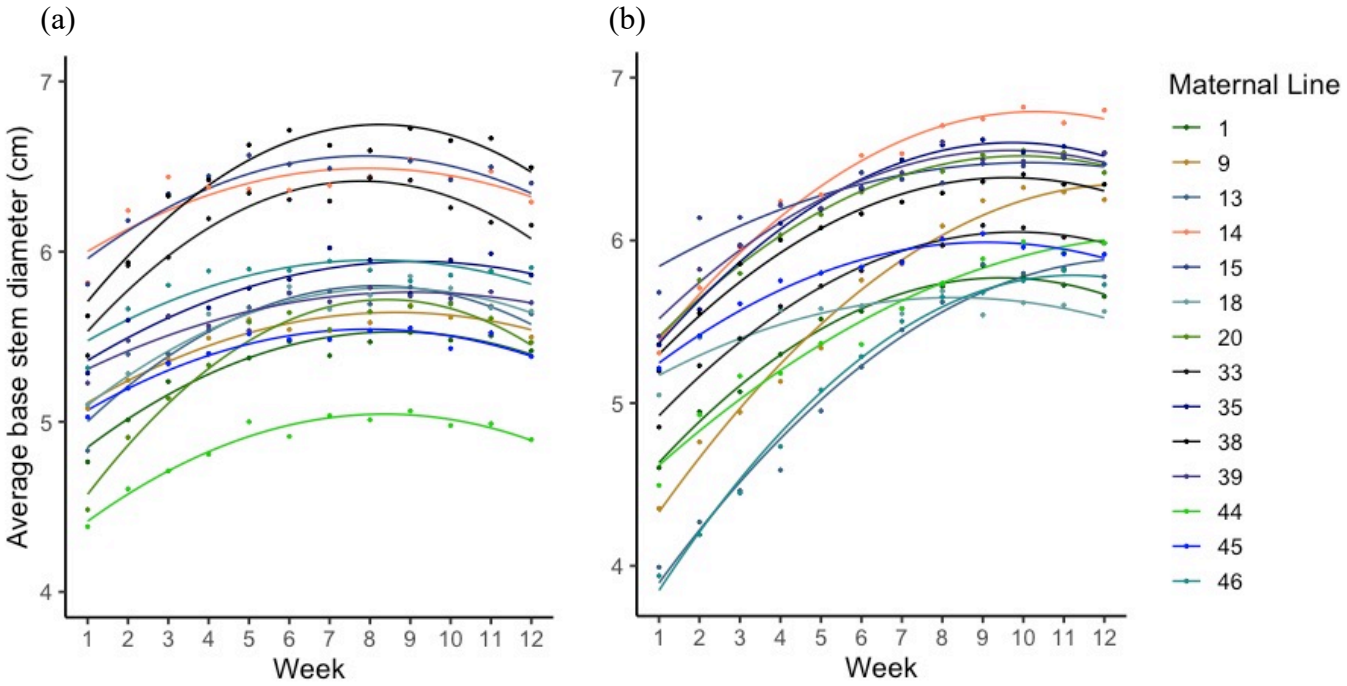

Figure B5 - Milkweed base stem diameter (cm) throughout the season in a (a) field common garden and a (b) greenhouse common garden. Maternal lines in the field common garden displayed genetic variation in base stem diameter growth rate and senescence rate (Week*Week*Maternal line, $F_{14, 2733} = 23.29$, $P < 0.0001$), as did maternal lines in the greenhouse common garden (Week*Week*Maternal line, $F_{14, 2744} = 16.33$, $P < 0.0001$). Points represent the mean height of 18 milkweeds of a maternal line for each week.